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SOME EFFECTS OF STEAM STERILIZATION ON PHYSICAL, CHEMICAL AND BIOLOGICAL RELATIONSHIPS OF SOILS.

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A THESIS

submitted to the University of Alberta
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Thesis
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11

TABLE OF CONTENTS.

		Page
Introduction		1
Review of Literature		3
Outline of Investigation		10
Methods of Analysis		14
Results and Discussion		22
Physical:	Mechanical Analysis	22
	Water Holding Capacity	27
	Shrinkage	29
	Sticky Point	41
	Capillary Rise	42
Chemical-biological:		
	Acidity	48
	Water Soluble Phosphorus	50
	Easily Soluble Phosphorus	52
	Nitrification	56
	Ammonification	66
	Sulfofication	76
	Ammonification by Pure Cultures	85
Summary		89
Acknowledgement		92
Bibliography		93

SOME EFFECTS OF STEAM STERILIZATION ON PHYSICAL, CHEMICAL AND BIOLOGICAL RELATIONSHIPS OF SOILS.

Introduction

The study of the complicated inter-relationships of soil micro-organisms (9) and plant diseases, and of soil micro-organisms and soil fertility, may be simplified by first studying the relationships of a few known organisms on sterilized soil devoid of its enormous population.

Each gram of soil commonly harbours millions of bacteria, thousands of fungi, Actinomycetes (27), some of each pathogenic to plants (7), algae, a smaller number of protozoa (8), nematodes, worms, insects, besides disease producing virus proteins, bacteriophages, mycophages, toxins, enzymes and various products of the micro-organisms including deliterious and stimulating substances. All these directly or indirectly have a bearing on plant diseases and soil fertility. The prevalence of certain organisms or the chemical changes brought about by them influence the activity of the other members of the soil microflora.

The study of the effects of known micro-organisms in sterilized soil necessitates a study of the physical and chemical-biological changes in the soil caused by sterilization, for the results may be misleading unless the changes due to sterilization are measured.

A great deal of work has been done since 1900 by various workers in England and the United States on "partial sterilization" by steam and chemicals. Their effects have been determined, so

that the findings obtained by these workers would in many cases apply to the soils as sterilized in this investigation. On the other hand, certain phases were investigated which the writer to date has not seen mentioned in soil literature.

Some physical properties of the four Alberta soils, including water relations, and chemical properties including their content of the three important plant elements, nitrogen, phosphorus and sulphur, as affected by sterilization and subsequently by microbial activities were investigated.

Review of Literature.

In experimental work soils are sometimes partially or completely sterilized in order to destroy certain groups or all of the living organisms normally found in the soil, with the object of studying their relation to the soil. In practice soils are sterilized in greenhouses to get rid of certain parasitic fungi, bacteria, actinomycetes and other undesirable organisms. Complete sterilization in practice is not aimed at (30) for there are many organisms which are beneficial to plant growth in that they improve the fertility of the soil by such processes as ammonification, nitrification, sulfofication and general decomposition of organic matter.

Of the different methods used to sterilize soils, dry heating, hot water, electricity, steam and chemicals have been tried, but so far the use of steam still gives the best and most complete sterilization.

In using dry heat there is always a danger of overheating certain parts of soil. Such soils become infertile and seedlings turn blue and grow very slowly if they grow at all (21).

Newhall and Nixon (33) investigated the method of electric pasteurization. They developed the "Ohio type" of pasteurizer in which the soil is heated by the resistance it offers to the electric current passed through the soil by special electrodes, and the "New York type", fitted with "pipe-type" spaced heaters placed at suitable intervals to impart heat to the soil by thermal conductance. In both they used a low temperature of 70 degrees C.

but it took 18 hours to heat up. It destroyed common plant pathogenes.

Practical sterilization is discussed by Senner (26), Bewley (21) and Newhall (30). Newhall concludes that steam sterilization leaves no toxic residues or water-logging and leaves the soil in good tilth (30).

Darbishire and Russell (34) in 1906 noted that in steam sterilized soils heated to 120 degrees C. the absorption of oxygen was greatly diminished but that in partially sterilized soils, heated to 95 degrees C., the rate of oxidation was considerably increased, due mainly to the activity of the surviving microorganisms. They studied the influence of partial sterilization, using volatile antiseptics and steaming at 95 degrees C. They found that the crop could get from heated soils three times as much nitrogen and twice as much phosphoric acid and potash as from unheated soils, and that the increase in plant dry matter was considerable. Such plants showed a higher percentage of nitrogen, phosphoric acid and potash in the dry matter. Inoculating partially sterilized soil with fresh soil caused a decreased plant growth compared to the uninoculated. This beneficial effect of partial sterilization alone extended over more than one crop so long as the new flora was left undisturbed, but by the addition of tap water the crop decreased. They believed that increased availability of the plant nutrients was connected with the modification of the bacterial flora, although they recognized that when the soils were sterilized by steam a certain amount of decomposition took place. They found that

volatile antiseptics also increased oxidation and the solubility of minerals and available phosphorus as judged by the amount taken up by the plant.

Russell and Hutchinson (11) found that heating the soil by steam to 125 degrees C. killed all the organisms present in the soil and caused a production of 5 p.p.m. of ammonia, but after that first production no further change took place. Heating by steam to 98 degrees C., or toluene, increased ammonification. For a few days there was a period of inactivation but this was followed by one of rapid change during which ammonia was produced in large quantities; lastly, a slow period of change set in and further production of ammonia was small. They concluded that organisms concerned with ammonification are not injured by heating to 98 degrees C. or by toluene but that these treatments destroy organisms which are inimical to ammonifying bacteria. Bacteria increased to great numbers following partial sterilization. This they attributed to the absence of protozoa. "Heat not only destroys the nitrifiers but brings about some change whereby the soil is rendered unsuitable for their development; they no longer act even when re-inoculated into the soil. It appears that an inhibitory substance is formed by heat." However, by the time the second crop was growing on heated soils, the nitrifying bacteria added developed abundantly, suggesting that the toxic body slowly disappears from the soil.

Lyon and Bizzell (14) found that nitrates remained low up to 14 weeks in steamed and re-inoculated soils and ammonia increased in sterilized re-inoculated and sterilized soils, due to

ammonifying bacteria from the air, since their soils were kept in sterilized fruit jars, afterwards loosely covered.

Baldwin (23) states that most of the organisms developing after partial sterilization are spore formers. Increase in ammonia is due to the activity of ammonifying bacteria, many of which are spore formers.

Waksman and Starkey (25) found that the formation of ammonia was directly dependent on the organic matter content of the soil and not correlated with increase in the numbers of bacteria when normal soils of different organic matter content were compared. Heating the soil caused bacteria to increase very markedly when re-inoculated into the soil. Toluene and heating influence greatly not only the development of bacteria and protozoa in the soil, but also that of Actinomycetes and especially fungi. They are represse at first but soon develop, especially when re-inoculated, and reach numbers greatly in excess of those of untreated soils. Ammonia accumulates in partially sterilized soils over the unheated soils, especially in soils rich in organic matter, but in poorer soils the rapid development of fungi may lead to a partial utilization of ammonia. Coleman (31) showed that marked differences exist in the activity of fungi regulated by the type of soil and the quality of organic matter. Chemicals beneficial to one group may be detrimental to another.

Koch (22) and Cutler (28) studied protozoa. Koch concludes that active protozoa are found in the soils only at high moisture content. Cutler (28) proved that protozoa were one of the factors concerned in keeping the numbers of bacteria below the level they might otherwise have attained.

Coleman, Lent and Kopeloff (17) sterilized soils at 80 degrees C. for one hour using dry heat on successive days up to 5 days. With intermittent sterilization using a low temperature, bacteria were almost completely decimated with but a slight change in the chemical constitution of the soil as judged by water soluble solids. Sterilization by steam increased the solids 10 times.

Schreiner and Lathrop (13) in 1912 found an increase in water soluble organic matter and in acidity (probably titrable acidity) in the steamed soil. At the same time ammonia and amines were formed. They found an increase in all organic constituents isolated from soils: xanthine, hypoxanthine, guanine, cytosine, arginine, di-hydroxystearic acid.

Pickering (12) found germination and plant growth were retarded in soils heated over 60 degrees C. up to 125 degrees C. (dry heat). He found more organic and inorganic matter in the heated soil extracts and this increased afterwards with time.

Lyon and Bizzell (16) found that steamed soil on standing up to 3 months, without plants, steadily decreased in water soluble organic matter content including ammonia and other soluble nitrogenous matter. This soluble organic matter rapidly decreased for several weeks in steamed soil re-inoculated with original soil (14). Plants made much better growth on steamed soils that were re-inoculated but didn't continue at the same rate throughout the season for they were finally exceeded by plants on uninoculated steamed soils. At maturity the latter had more dry matter.

Baldwin (23) found a consistent increase in acid soluble phosphorus after sterilization but no figures are given. His determination was made on samples of sterilized greenhouse soil.

McCool, referred to by Newhall (30), found soluble manganese may be increased materially by steam sterilization. A lower water holding capacity in steam sterilized soil has been reported (30) and (12).

Various chemicals have been tried as soil antiseptics. When used in large doses they bring about almost complete sterilization for a time, but when the chemical is used up the organisms regain their activity.

Carbon bisulphide, toluene, chloroform and formalin in small doses produced conditions similar to partial sterilization (19) but mercuric chloride, copper sulphate and thymol did not increase productiveness though very small doses stimulated the activity of the microflora (34).

The use of volatile antiseptics, in partial vacuum or under a combination of heat and pressure, if repeated for three successive days would achieve complete soil sterilization without involving radical alterations in the chemical constitution of the soil (17). According to Darbishire and Russell (34), no amount of solution will bring every particle of soil in contact with the disinfectant, therefore it is reasonable to suppose that mixing a powdered chemical will not distribute it thoroughly through the soil.

Matthews (19) found bacterial numbers were increased for the first few days following application of antiseptics, the increases varying in the same direction as the molecular weight and heat of combustion of the antiseptic. After a sudden rise in numbers to the maximum there was a gradual return to normal which, she concluded, was due to the feeding effect of the antiseptic.

Aliphatic compounds gave quicker and smaller rises than aromatic, and an introduction of the methyl group into the benzene ring lessened toxicity but chlorine and the nitro group increased both toxicity and stability of compound.

Buddin (18) found phenol and its derivatives were effective if used in high enough doses. Small doses, M/200 up to M/50, caused a high rise in bacterial numbers but M/10 to M kept the soil protozoa and bacteria in an inactive condition for 75 days. Phenol, M/50 to M, keeps protozoa and bacteria inactive for 75 days. Dixon, Annie (20) studied the effects of phenol, carbon-bisulphide and heat on protozoa and found that all strengths of phenol below 1.8 percent showed the presence of protozoa in time and that heating the soil in a steamer for 30 minutes is usually sufficient to kill the protozoa. Phenol, she found, had greater lethal effect on protozoa than carbon-bisulphide.

Calcium cyanamide in small doses increased the activity of soil organisms as partial sterilization (24), but in large doses had a depressing effect. The amount depends on the nature and hydrogen ion concentration of the soil. It controlled "damping off" and prevented the damage due to Plasmodiophora brassicae. Doran (32) compared formaldehyde, acetic acid and pyroligneous acid as soil disinfectants and found pyroligneous acid a cheap, safe, effective disinfectant for "damping off." Cresylic acid was as good as formaldehyde; it increased the yield, though less than steaming (21). Carbon bisulphide and formalin together gave the best and most complete chemical sterilization, but it is too expensive (30).

Outline of Investigation

The investigation was divided into two phases, first, the physical, and second, the chemical-biological changes effected by steam sterilization in four Alberta soils: Edmonton sod, a black park loam; Vegreville cultivated, black park loam also but more sandy in texture; Gros Ventre brown prairie loam, and Fallis gray wooded silty loam. The first two soils mentioned were high in organic matter while the last one was very low in this respect.

In the study of the physical properties of these soils the non-sterilized were compared with the sterilized with respect to mechanical analysis, water holding capacity, shrinkage, sticky point, and capillary power. Since soil moisture relations affect greatly the ultimate productive power of the soil, certain differences in sterilized soils may be due to purely physical factors.

The chemical-biological phase included the study of soil acidity, nitrification, ammonification, sulfofication, easily soluble phosphorus and ammonification by pure cultures. These processes in certain ways are indicative of the microbiological activity and the productive power of the soil.

In the study of the physical properties only two treatments were compared, -- the non-sterilized with the sterilized soil. In the chemical-biological, three treatments, the non-sterilized, sterilized and sterilized re-inoculated were compared in order to determine what changes were caused by sterilization alone,

and how the microbial activity responded to these changes, as compared to normal soils.

For the study of the physical properties, the soil was sterilized when needed, in one gallon crocks. In the study of the second phase the soils were sterilized in Erlenmeyer flasks and incubated in a dark control chamber at room temperature (23 degrees C. to 27 degrees C.)

Two nitrification experiments were conducted. The first was started in the fall of 1936 and carried on for 12 weeks. Nitrates were determined one day after setting up the experiment, then every 2 weeks for 12 weeks. The second, started in April 1937, was carried on for 39 weeks and nitrates were determined at the end of 6 days, 6 weeks, 12 weeks, 14 weeks ---------- 39 weeks.

The plan followed was the same in both. In the first, a total of 84 (200 cc.) flasks, each containing 50 grams of soil (water-free basis), were incubated. This consisted of 7 flasks of each treatment, making 21 flasks of each soil, namely, 7 of non-sterilized, 7 sterilized and 7 of sterilized and re-inoculated with one per cent of the original soil. One flask of each treatment was taken out for each determination. Duplicates were taken out of the same flask in order to decrease the amount of apparatus required.

The second nitrate experiment was made up of 88 (200 cc.) flasks and 44 tumblers incubated as in the above experiment. The sterilized and the sterilized re-inoculated were incubated in flasks while the non-sterilized were incubated in tumblers with

perforated covers. This experiment was made up of 11 samples of each treatment for each soil. For each determination one flask or tumbler was taken out and duplicates taken from it. Nitrates were determined in the second experiment right after sterilization, then at the end of 6 days, 6 weeks, 12, 14, 16, 18, 20, 26, 30 and 39 weeks.

The ammonification experiment was outlined following the same plan. A total of 120 (200 cc. Erleumeyer) flasks, each containing 50 grams of soil (water-free) were incubated as above. This experiment included 30 flasks of each soil, that is 10 flasks of each treatment. Ammonia was determined 3 days, 6 days, 2 weeks, 4, 6, 8, 10, 12, 16 and 20 weeks after setting up the experiment. Before determining ammonia, 2 grams of soil (water-free) out of each flask were taken for easily soluble phosphorus determination.

In the sulfofication experiment 3 treatments of each of the 4 soils were again included. A total of 168 samples (each 100 grams water-free) were incubated, one-third of them in tumblers and two-thirds in flasks with cotton plugs as for nitrates and ammonia. The flasks contained the sterilized and the sterilized re-inoculated soils. Out of the 14 samples of each treatment, 2 were taken out each time for analysis, duplicates being taken from separate flasks and tumblers. Water soluble sulphates were determined right after sterilization and upon 2 weeks, 4, 8, and 12 weeks incubation.

In the experiment with pure cultures of Ophiobolus, an antagonistic fungus (no. 32), and a non-antagonistic fungus (no. 3), 36 flasks of Edmonton sod were sterilized and 12 in-

oculated with each organism. These were incubated in a dark control chamber at a temperature of 23 to 27 degrees C.

Methods

Soil samples, collected in the summer of 1936, were air-dried and stored in large glass containers in the laboratory. These glass containers were replenished from time to time as needed with soils brought in from the shed where the bulk of each sample, except the Edmonton, was stored, while in the case of the Edmonton soil a second sample was taken from the same field in the fall of 1937.

All soils were put through a 2 mm. sieve before setting up any experiment, to secure uniformity by excluding extraneous matter, roots, stones and especially coal-like inert matter from Fallis gray wooded soil.

Before sterilization the soils were brought to optimum moisture and allowed to stand half an hour to one hour to thoroughly wet the particles, except in the capillary experiment when the soils were moistened for two days before sterilization.

The optimum moisture used for Edmonton black park soil was 36 per cent; for Vegreville black park soil 30 per cent; for Gros Ventre brown prairie soil 22 per cent and Fallis gray wooded 19 per cent.

Table I shows the hygroscopic moisture, optimum moisture and a few other physical characters of the soils worked with.

Sterilization was done by steaming the soil once for 45 minutes at full pressure (15 to 17½ pounds of steam) in the autoclave, cooling one day and sterilizing the second time, for

30 minutes again at full pressure (approximately 120 degrees C.)

After sterilization the soils were cooled and half of the sterile flasks were re-inoculated with ½ per cent to 1 per cent of the original soil for nitrification, ammonification and sulfofication experiments. In other experiments where the sterilized soils were compared with the non-sterilized soils for physical properties and for phosphorus without incubation, no re-inoculation treatments were included.

For mechanical analysis, Bŏyoucos method (1) was used, but instead of starting with oven dry soil, as would ordinarily be done, moist samples were weighed. This was done because, in order to sterilize the soil properly without overheating at the bottom or along the sides of the container, and thereby causing drastic changes, the soil must be quite moist; therefore, all soils were brought to optimum moisture prior to autoclaving. The non-sterilized soils had the same amount of moisture added as the sterilized, and were kept moist for the same length of time. It was thought that following sterilization, drying may affect the soil colloidal content, and thereby alter changes induced by steam; therefore, moist samples were worked with. Moisture was determined on separate samples and all calculations were based on water-free weight. Since it was much more difficult to weigh accurately moist samples, due to the rapid loss of moisture, a greater error had to be contended with. To each sample 5 cc. of sodium oxalate and sodium silicate were added. All soils were stirred for 15 minutes except Fallis soil which

was stirred for 20 minutes; in other respects the usual procedure was followed.

Some seven determinations were made by the pipette method, which consisted of drawing out aliquots of 20 cc. after settling for 50 seconds, from a depth of 10 cms. below the surface of the liquid in the "Bouyoucos" cylinder; after 98 seconds from a depth of 20 cms., and after 80 minutes from the same depth. These aliquots were evaporated in small dishes at 100 degrees C., cooled and weighed and the percentages of clay, silt, and sand computed by difference.

Real specific gravity was found on 10 grams of oven dry soil weighed in a 25 cc. volumetric flask filled with water to the mark.

$$\text{Real specific gravity} = \frac{\text{Weight of soil in grams}}{\text{Volume of soil in cc.}}$$

Water holding capacity was found by the funnel method using 50 grams of soil. Readings were taken when drainage stopped. In certain determinations readings were made at 15 minutes, 1 hour and 2 hours.

Shrinkage was determined by a modification of Haines (2) method, by displacement of mercury. The soils were brought to optimum moisture, half of each was sterilized twice in the manner mentioned, then both the non-sterilized and the sterilized soils were air-dried and passed through a 100 mesh sieve. Capillary moisture still retained was determined. Into 50 grams (air-dry) soil placed on a glass plate, a measured amount of moisture from a graduated cylinder was incorporated by

means of a spatula until the sticky point was almost reached. It was then kneaded, shaped by hand and moistened more especially at the ends, to prevent cracking. The wet soil cylinder was weighed and its volume determined by placing it in a glass centrifuge tube, held erect, and then filling the tube with mercury and levelling off with a microscope slide. The buoyant effect was overcome by placing a perforated rubber stopper below the level of mercury. Weight and volume of the soil cylinder were measured at intervals of drying first at room temperature, later in an over at 100 degrees C. and finally after oven drying for 2 days at 110 degrees C. All data were reduced to the basis of 1 cc. oven dried soil. Some difficulty arises, using this method, in finding the first volume if the soil cylinder is too wet, as the buoyant force of mercury compresses the soil and distorts the soil cylinder; also, small bubbles of air often adhere. But with a little drying these difficulties can easily be overcome and the volume measured accurately.

The sticky-point was determined on the four sterilized and non-sterilized soils by a somewhat arbitrary method. Air-dry 50 gram samples were moistened with a measured amount of water and the moisture worked into the soil with a spatula, on a glass plate as for shrinkage, until the sticky-point was nearly reached. This was judged by the appearance of wetness. Then the wet soil was kneaded by hand and a little more moisture worked into it until the soil became sticky as judged by the adhesion to the palm of the hand and the force required to tear away the soil cake after squeezing in the hand.

Capillary rise in the four sterilized and the non-sterilized soils was determined by the ordinary method. Glass tubes 3 to 4 feet long, 1 1/6 to 1 1/8 inches in diameter, wrapped at one end with four plies of cheese cloth, were filled with soil and immersed vertically in a pan containing water maintained at a constant level. The soils were brought to optimum moisture, allowed to stand two days to become uniformly wet, and the lost moisture was made up. Half of each soil was sterilized as described above, then all the soils were spread uniformly on paper to dry for 5 days. Moisture was determined before the tubes were filled. The soils were put through a 2 mm. sieve and the large granules broken up gently. The tubes were filled by pouring the soil through a funnel suspended at a uniform height, care being exercised to secure an even distribution of the large and small soil particles. The soil was packed by tapping gently the sides of the tubes with a rubber pestle until the soil column moved down a definite distance (approximately 3½ inches). From the time water was added readings of the capillary rise above the water level were taken at 5 minutes, 15 minutes, 1 hour, 2, 3, 4, 12 and 24 hours and thereafter every day for 42 days, and at the longer intervals until 61 days from the start.

The pH values were determined by the quinhydrone electrode method. One day after sterilization, moist samples equivalent to 5 grams of water-free soil were diluted with 20 cc. of distilled water and pH values read at 1, 5, 15 and 30 minutes.

Water soluble phosphorus was determined colorimetrically on

1:5 water extracts of soil filtered through Berkefeld filters by Parker's method (3). Moist samples equivalent to 50 grams water-free soil were extracted for one hour on a shaker and 25 or 50 cc. filtrate evaporated in porcelain dishes to which 1 cc. of 10 percent $Mg(NO_3)_2$ was added. Organic matter was oxidized and ignited off in the electric muffle. The ash was dissolved with 1:20 HCl, made up to required volume and read with ammonium sulphuric acid molybdate and stannous chloride according to the usual procedure. A few samples were determined without ignition, in which case the same procedure was followed in that all reagents were added in the same amounts as for the above only that $Mg(NO_3)_2$ was oxidized first and ignited off before the filtrates were evaporated.

Easily soluble phosphorus (acid soluble at pH 3.0) was determined colorimetrically by Truog's (4) method. Moist samples of soil equivalent to one gram water-free were extracted by shaking one hour, with 200 cc. buffered pH 3.0 sulphuric acid solution, adjusted when necessary by the addition of 1 to 7 drops of N sulphuric acid in order to maintain this reaction. The pH values were read before and after extraction with the potentiometer. A few soil extracts after filtering through paper were highly colored and such were cleared by means of activated charcoal. Using 25 cc. filtrate, blue color was developed with ammonium molybdate sulphuric acid solution and stannous chloride.

Easily soluble phosphorus upon incubation was determined on soils taken from the flasks incubated for ammonification just before determining ammonia. The flasks were weighed up and

brought to optimum moisture, if they had lost any moisture, cultivated thoroughly and duplicate moist samples equivalent to one gram (water-free) soil were weighed out for phosphorus analysis and put into stoppered shaker bottles for later determination. Some difficulty in weighing moist samples was encountered due to rapid drying. This was partly avoided by making rapid weighings.

Nitrates were determined colorimetrically by the phenol-disulphonic method as modified by H. J. Harper (5) in which one to five water extractions were analysed.

Ammonia was determined by the McLean and Robinson (6) method of leaching 25 grams of soil with 500 cc. N. sodium chloride solution and distilling the soil extract with magnesium oxide, collecting ammonia in standard acid and titrating the excess with a standard base using M.R. Distillation was carried on for $\frac{3}{4}$ to 1 hour until approximately 250 cc. distillate were collected.

To find if the magnesium oxide added in the first distillation upon standing one day in the solution had a breaking down effect on the dissolved organic matter, thus further liberating ammonia, 200 cc. of distilled water were added and a second distillation was carried out.

Water soluble sulphates were determined gravimetrically by precipitation as barium sulphate. Extractions were made by shaking for one hour 100 grams of soil with 250 cc. of water in the first two determinations, and with 500 cc. in all the others. The soil extracts were filtered through Berkefeld filters, evaporated to dryness with $Mg(NO_3)_2$ and ignited in an electric muffle

at 600 degrees C. for ½ hour.

In the pure culture experiment ammonia was determined by the same method referred to above, that of McLean and Robinson.

In all cases where the soil was incubated, the flasks were taken out of the chamber every week or ten days, weighed and the loss of moisture determined. The flasks were watered with sterile water by means of a sterile pipette, flaming the mouth of the flask each time the cotton plug was removed. By means of a stirring rod which was placed in each flask through the cotton plug, the soils were cultivated after being watered. It was found difficult to keep the cotton plugs in good condition, especially after prolonged incubation they became loose.

RESULTS AND DISCUSSION.

PHYSICAL: Mechanical Analysis.

Mechanical analyses were made on 25 samples, of which the results of 16 are represented in graph 1 and illustrative data are supplied for each soil in table 2. They show the trends obtained when sterilized and non-sterilized soils are compared.

According to graph 1, taking the silt limits as 0.002 mm. to 0.05 mm. in diameter of particles, the Edmonton black park soil should be classed as loam to silt loam, the Vegreville black park soil as sandy loam (probably fine sandy loam), the Gros Ventre brown prairie soil as loam, and the Fallis gray wooded soil as silt loam.

Negative results were obtained with respect to the differences between sterilized and non-sterilized, at least down to 0.002 mm. in diameter of particles.

With the pipette method somewhat lower percentages were obtained for silt, lower for clay, and somewhat higher percentages for the sand fractions.

Table 1 - Physical characters of the four Alberta soils.

	Hygroscopic moisture %	Moisture holding capacity in %	Optimum % moisture	Optimum % of water holding	Treatment	Specific gravity	Sticky point % moisture
Edmonton black park (loam to silt loam)	5.96	76 72	36	47.3	Non-ster. Ster.	2.34 2.40	60 58
Vegreville black park (sandy loam to fine sandy loam)	4.52	71 71	30	42.3	Non-ster. Ster.	2.51 2.54	52 48
Gros Ventre brown prairie (loam)	2.13	75 74	32	43.2	Non-ster. Ster.	2.32 2.33	50 46
Fallis gray wooded (silt loam)	1.22	59.5 58.5	19	30.0	Non-ster. Ster.	2.54 2.51	33 33

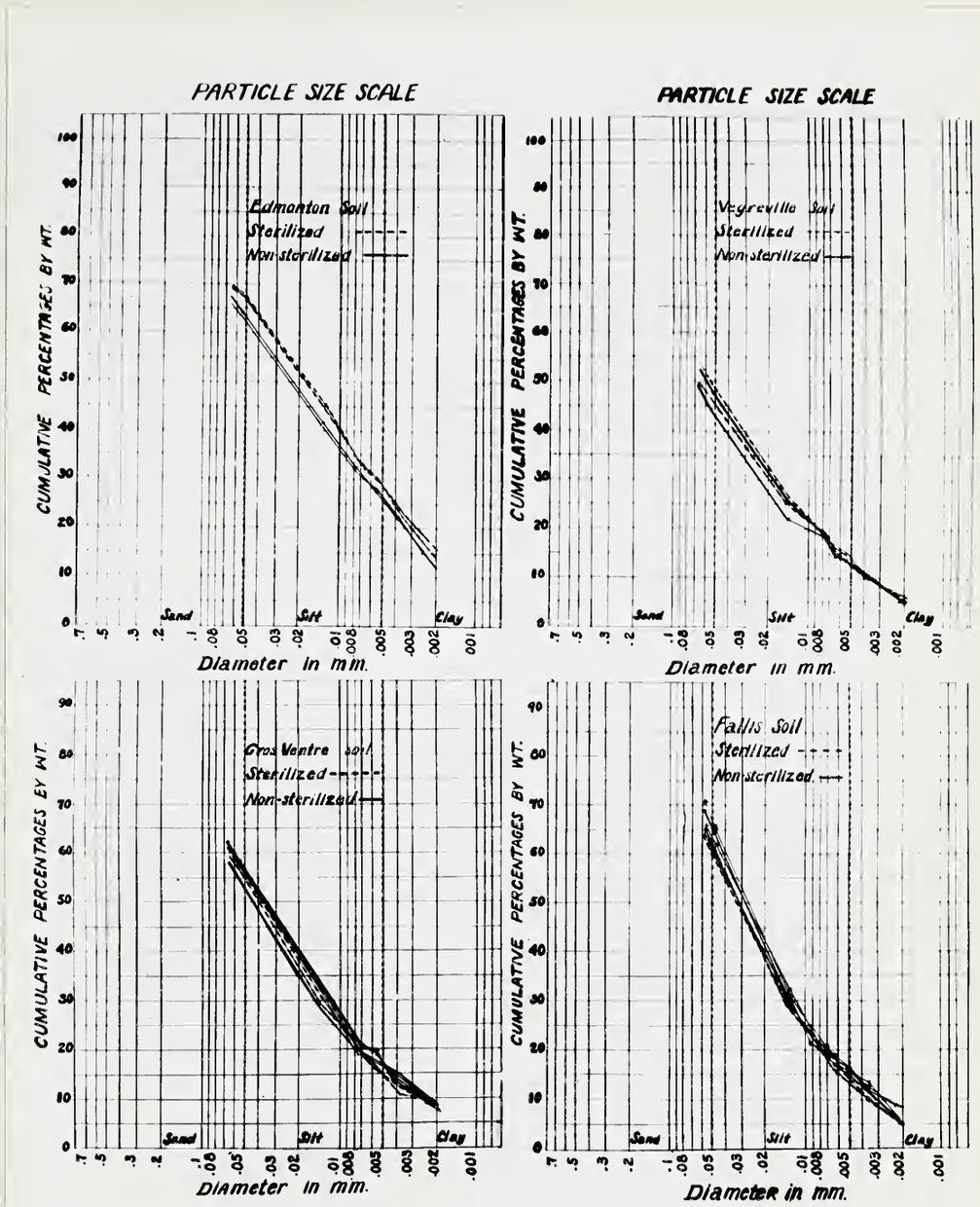
Table 2 - Thermal conductivity of the four Alberta soils.

Table 2 - Mechanical Analysis.

Soil	Treatment	Hydrometer Rdg.	Time	% finer than	Particle diam. in mm.
Edmonton (Black) loam	Non-ster.	23.5	40 sec.	67.30	.066
		22.5	60 "	64.44	.055
		14.5	15 min.	41.53	.015
		11.66	1 hr.	33.41	.0076
		9.5	2 "	27.21	.0055
		8.0	4 "	22.91	.0039
		5.5	9.5 hr.	15.75	.0025
Edmonton (Black) loam	Ster.	26.5	40 sec.	68.9	.062
		26.0	60 "	67.6	.052
		17.5	15 min.	45.5	.014
		13.0	1 hr.	33.8	.0073
		11.5	2 "	29.9	.0052
		9.0	4 "	23.4	.0037
		7.0	9.5 hr.	18.2	.0024
Vegreville black park	Non-ster.	19.0	40 sec.	52.71	.066
		17.0	60 "	47.16	.055
		9.5	15 min.	26.35	.015
		6.5	1 hr.	18.03	.0076
		6.0	80 min.	16.64	.0066
		5.5	2 hrs.	15.26	.0054
		4.0	4 "	10.10	.0038
		2.0	13 "	5.55	.0022
Vegreville black park	Ster.	19.0	40 sec.	52.54	.066
		18.0	60 "	49.79	.054
		9.5	15 min.	26.28	.015
		7.0	1 hr.	19.36	.0076
		6.0	80 min.	16.60	.0066
		5.5	2 hrs.	15.21	.0054
		4.0	4 "	11.06	.0039
		2.5	13. ""	6.91	.0022
Gros Ventre brown prairie	Non-ster.	28.5	40 sec.	58.01	.065
		27.0	60 "	54.97	.054
		14.0	15 min.	28.50	.0155
		10.0	1 hr.	20.36	.008
		8.0	2 hrs.	16.35	.0054
		7.5	4 "	15.27	.0040
		5.0	11¾ "	10.18	.0024
Gros Ventre brown prairie	Ster.	30.0	40 sec.	61.32	.062
		27.0	60 "	55.19	.055
		14.0	15 min.	28.62	.015
		9.0	1 hr.	18.40	.0076
		8.75	80 min.	17.88	.0055
		6.66	4 hrs.	13.61	.0039
		5.5	11¾ "	11.24	.0023

Table 2 continued - Mechanical analysis.

Soil	Treatment	Hydrometer Rdg.	Time	% finer than	Particle diam. in mm.
Fallis gray wooded	Non-ster.	32.7	40 sec.	65.89	.056
		31.0	60 "	62.47	.047
		16.0	15 min.	32.24	.0135
		9.0	60 m"	18.13	.007
		8.0	2 hrs.	16.12	.005
		6.5	4 "	13.10	.0036
		3.0	11 "	6.05	.0022
Fallis gray wooded	Ster.	33.5	40 sec.	65.29	.057
		31.0	60 "	60.42	.047
		14.0	15 min.	27.29	.014
		9.0	1 hr.	17.54	.007
		8.7	80 min.	17.06	.0062
		8.0	2 hrs.	15.59	.0051
		6.5	4 "	12.67	.0036
		3.0	11 "	5.85	.0022



Graph 1. Mechanical analysis of non-sterilized and steam sterilized Edmonton and Vegreville black park soils, Gros Ventre brown prairie soil, and Fallis gray wooded soil.

Water Holding Capacity.

Most of the work on water holding capacity of sterilized and non-sterilized soils was done with Edmonton soil although the other three soils were tried. Table 3 illustrates the type of results obtained, using the funnel method. The averages of 16 determinations with each treatment of Edmonton soil were 72 percent for the sterilized and 73 percent for the non-sterilized soil. These figures are not included in table 3. The figures that appear in table 3 for Edmonton soil were obtained in a subsequent determination using another batch of sterilized soil. The results show that the sterilized soil has a slightly lower water holding capacity, but the differences were not very definite and were not obtained in every case. The variation between different determinations was often more than 4 percent, therefore the differences found cannot be taken as significant. Newhall (30) and Pickering (12) report that sterilized soils have a lower water holding capacity, but they also hold water for a longer time (12).

Table 3 - Percent water holding capacity of the four Alberta soils.

Soil	Treatment	cc's. water retained by 50g. soil after drainage stopped. 5 min. after	1 hr. after	2 hr. after	% water holding capacity based on moisture retained after drainage stopped. 5 min. after	1 hr. after	2 hr. after	Ave. H_2O holding capacity.
Edmonton black park	Non-sterilized	38.0	38.0	38.0	76.0	76.0	76.0	
		38.2	38.0	38.0	76.4	76.0	76.0	76%
	Sterilized	36.6	36.0	36.0	73.2	72.0	72.0	
		36.0	36.0	36.0	72.0	72.0	72.0	72%
Vegreville black park	Non-sterilized	35.5	35.5	35.5	71.0	71.0	71.0	
		35.0	35.5	35.5	70.0	71.0	71.0	71%
	Sterilized	34.8	35.8	35.5	69.6	71.6	71.0	
		35.2	36.0	36.0	70.4	72.0	72.0	71%
Gros Ventre brown prairie	Non-sterilized		38.5	37.0		77.0	74.0	
			38.0	37.0		76.0	74.0	75%
	Sterilized		36.0	36.0		72.0	72.0	
			38.0	38.0		76.0	76.0	74%
Fallis gray wooded	Non-sterilized		29.5	29.5		59.0	59.0	
			30.0	30.0		60.0	60.0	59.5%
	Sterilized		29.5	29.5		59.0	59.0	
			29.0	29.0		58.0	58.0	58.5%

Table 5 - Percent water holding capacity of the four alterna soils.

Soil	Treatment	cc's. water retained by 50g. soil after drainage stopped. 5 min. after	1 hr. after	2 hr. after	% water holding capacity based on moisture retained after drainage stopped. 5 min. after	1 hr. after	2 hr. after	Ave. H_2O holding capacity
Sunnyvale block	Non-sterilized	35.0	38.0	38.0	70.0	70.0	70.0	
		35.2	35.0	36.0	70.4	70.0	70.0	70%
	Sterilized	36.0	36.0	36.0	72.0	72.0	72.0	
		36.0	36.0	36.0	72.0	72.0	72.0	72%
Yegreville block	Non-sterilized	35.5	35.5	35.5	71.0	71.0	71.0	
		35.0	35.5	35.5	70.0	71.0	71.0	71%
	Sterilized	35.2	36.0	36.0	70.4	72.0	72.0	71%
Gros Ventre brown	Non-sterilized	36.5	37.0		73.0	74.0		
		36.0	37.0		70.0	74.0		73%
	Sterilized	36.0	36.0		72.0	72.0		
		36.0	36.0		70.0	70.0		74%
Fallis grey	Non-sterilized	29.5	29.5		59.0	59.0		
		30.0	30.0		60.0	60.0		59.5%
	Sterilized	29.5	29.5		59.0	59.0		
		29.0	29.0		58.0	58.0		58.5%

Shrinkage.

The results of the shrinkage experiment are represented by graphs 2 and 3, and data are supplied in table 4. They show that during the early stages of drying there was a decrease in the volume of the soil, proportional to the amount of moisture lost.

This decrease, represented graphically, is shown by the slant of the curve at an angle of approximately 45 degrees to the abscissa. It is indicated well in graph 3 in Fallis soil in the non-sterilized, from 0.45 cc. to 0.35 cc. moisture content per cc. of soil; and in the Fallis sterilized from 0.45 cc. to about 0.33 cc. of moisture. The same trend is shown by Gros Ventre soil from 0.6 cc. to 0.45 cc. loss in moisture by the non-sterilized, and from 0.61 cc. to 0.48 cc. by the sterilized. No doubt a sharper break would have been obtained had another reading been taken at about 0.4 cc. loss of moisture per cc. of soil.

Examination of graph 2 shows the same trend, but for a short distance; in the Edmonton soil from 0.75 cc. to 0.6 cc. and possibly to 0.4 cc. loss of moisture by both the non-sterilized and sterilized soil. Vegreville non-sterilized follows this trend from 0.8 cc. to 0.55 cc. and possibly to 0.5 cc. of moisture per cc. of soil.

There was a sudden change in the direction of the curves in Fallis soil from 0.35 cc. moisture in the non-sterilized and 0.33 cc. in the sterilized up to the last stage of drying. The curves become almost parallel to the base (abscissa). This stage of drying is accompanied by a large loss in moisture but hardly any shrinkage in volume; at least the volume of moisture lost far

exceeds the change in volume of soil. This "residual" shrinkage in the non-sterilized Fallis amounted to approximately 0.02 cc. decrease in volume for 0.35 cc. of moisture lost, and in the sterilized approximately 0.025 cc. for 0.33 cc. of moisture lost.

The break between the "normal" shrinkage and the "residual" shrinkage is not so sharp in the Gros Ventre soil; probably it would have been, had a reading been made at about 0.37 cc. moisture content per cc. of soil. However, taking the points plotted at 0.23 to 0.25 cc. of moisture, which is well in the region of the residual shrinkage for this soil, the volume of the soil, at 0.24 cc. of moisture in the non-sterilized is approximately 1.02 cc., while in the sterilized at the same moisture content it is approximately 1.03 cc. to 1.04 cc. Again this is a very small difference, but the shrinkage is greater in the sterilized soil.

In graph 2 the Vegreville soil shows a more gradual change instead of a sharp break. This is indicated by the points plotted between 0.1 cc. and 0.65 cc. of moisture in the sterilized, and between 0.15 cc. and 0.56 cc. in the non-sterilized. A slightly greater shrinkage of about the same magnitude as in the previous two soils appears in the case of the sterilized soil, below a moisture content of approximately 0.25 cc.

The Edmonton soil shows the same trends as the Vegreville soil discussed.

From graphs 2 and 3 it appears that "residual" shrinkage is smallest in Fallis gray wooded soil and greatest in the Edmonton soil with the Vegreville following. This indicates that soils high in organic matter differ from the gray wooded soil which is low in this respect, and agrees with the findings of Haines (2)

who worked with Rothamsted soils, that the highest "residual" shrinkage of 18.2 percent was found in peat soil at 50.5 percent moisture content, and only 2.2 percent in the Rothamsted loam at 18.7 percent of moisture.

When these soils are compared for the differences in shrinkage effected by sterilization, slightly greater shrinkages were found in almost all soils in the case of the sterilized, but when these differences are statistically analysed they are found to be hardly significant.

The expression "based on 1 cc. water-free soil" has been used in the above rather loosely. Actually the 1 cc. of water-free soil included some air in the pore spaces between the oven dried soil particles.

Likewise, for example, the term 1.1 cc. soil volume actually would mean the volume which the soil and air and water occupied; that is, the wet volume of the soil measured, divided by the volume the same amount of soil occupied when oven dried.

Table 4 - Shrinkage data for non-sterilized and steam sterilized Edmonton, Vegreville, Gros Ventre and Fallis soils.

Edmonton sod Non-sterilized

	Wt. of soil cylinder in grams.	Vol. of soil cyl. in cc's (V)*	Wt. of water in grams.	% water by wt.	Vol. soil reduced $\frac{(V)}{(Vo)}$**	Water lost by 1 cc. oven dry soil $\frac{(wt.\ water)}{Vo}$
Sample 1.	70.37	43.2	23.05	48.5	1.340	.6900
	67.27	40.1	19.95	41.7	1.240	.6050
	59.74	35.9	12.42	26.1	1.084	.3730
	51.75	34.1	4.43	9.3	1.025	.1340
	47.68	33.0	.36	.75	1.000	.0109
	47.32	33.0	.00	.0	1.000	.0000
Sample 2.	71.68	44.3	24.35	51.5	1.340	.7380
	68.92	42.0	21.59	45.8	1.270	.6530
	60.75	36.1	13.42	28.5	1.090	.4070
	52.42	33.6	5.09	10.7	1.020	.1540
	47.82	33.0	.49	1.03	1.000	.0148
	47.33	33.0	.00	.0	1.000	.0000
Sample 3.	71.50	44.5	24.23	51.5	1.350	.7350
	68.27	41.4	21.00	44.5	1.255	.6370
	58.28	35.8	11.01	23.5	1.084	.3340
	51.28	33.6	4.01	8.5	1.019	.1215
	48.04	33.0	.77	1.84	1.000	.0233
	47.89	33.0	.62	1.31	1.003	.0187
	47.27	33.0	.00	.0	1.000	.0000

* Volume moist
** Volume oven dry

Edmonton Sterilized

	Wt. of soil cylinder in grams	Vol. of soil cyl. in cc's (V)	Wt. of water in grams	% water by wt.	Vol. soil reduced $\frac{(V)}{(Vo)}$	Water lost by 1 cc. oven dry soil $\frac{\text{wt. water}}{Vo}$
Sample 1.	70.59	43.9	23.62	49.6	1.348	.7250
	66.41	40.2	19.44	40.9	1.231	.5960
	56.79	34.8	9.82	20.6	1.069	.3010
	50.76	33.0	3.79	7.9	1.011	.1163
	47.74	32.6	.77	1.6	1.000	.0236
	47.56	32.6	.59	1.2	1.000	.0181
Sample 2.	73.12	45.6	26.12	55.5	1.395	.8012
	69.62	40.2	22.62	48.2	1.230	.6939
	59.94	35.7	12.94	27.5	1.095	.3969
	51.55	33.0	4.55	9.2	1.012	.1396
	47.98	32.6	.98	2.1	1.010	.0300
	47.68	32.6	.68	1.4	1.000	.0212
	47.00	32.6	.00	.0	1.000	.0000
Sample 3.	70.82	44.0	24.03	51.4	1.362	.7440
	67.00	40.2	20.21	43.2	1.245	.6257
	57.35	34.7	10.56	22.8	1.074	.3269
	50.71	33.0	3.92	8.4	1.022	.1214
	47.75	32.5	.96	2.0	1.006	.0297
	47.39	32.5	.66	1.3	1.006	.0186
	46.79	32.3	.00	.0	1.000	.0000

Vegreville Non-Sterilized

	Wt. of soil cylinder in grams	Vol. of soil cyl. in cc's (V)	Wt. of water in grams	% water by wt.	Vol. soil reduced $\frac{(V)}{(Vo)}$	Water lost by 1 cc. oven dry soil $\frac{wt.water}{Vo}$
Sample 1.	69.35	37.1	23.75	51.5	1.408	.8317
	66.90	39.1	21.3	46.6	1.308	.7460
	61.70	35.1	16.1	36.8	1.174	.5638
	54.30	31.8	8.7	19.1	1.064	.3047
	52.50	30.5	6.9	15.1	1.020	.2416
	50.60	30.3	5.0	10.9	1.013	.1751
	45.70	29.9	.1	.2	1.000	.0031
	45.60	29.9(Vo)	.0	.0	1.000	.0000
Sample 2.	72.95	46.4	26.15	55.9	1.432	.8083
	69.60	40.3	24.3	52.0	1.346	.7500
	66.50	39.2	19.7	42.1	1.210	.6080
	59.10	34.9	12.3	26.3	1.077	.3796
	55.60	33.6	8.8	18.8	1.037	.2716
	53.50	33.2	6.7	14.3	1.025	.2021
	47.10	32.4	.3	.6	1.000	.0093
	46.80	32.4(Vo)	.0	.0	1.000	.0000
Sample 3.	77.10	49.3	27.7	56.2	1.373	.7716
	72.20	42.3	20.4	41.4	1.180	.5700
	62.30	38.9	12.9	26.2	1.083	.3594
	58.00	37.3	8.6	17.5	1.039	.2396
	54.90	36.9	5.5	11.2	1.028	.1532
	49.50	36.1	.1	.2	1.006	.0010
	49.40	35.9(Vo)	.0	.0	1.000	.0000

Vegreville sterilized

	Wt. of soil cylinder in grams	Vol. of soil cyl. in cc's (V)	Wt. of water in grams	% water by wt.	Vol. soil reduced $\frac{(V)}{(Vo)}$	Water lost by 1 cc. oven dry soil $\frac{(wt.water)}{Vo}$
Sample 1.	72.43	46.2	26.05	56.3	1.324	.7464
	68.88	43.1	22.50	48.7	1.235	.6447
	58.02	37.5	11.64	25.2	1.075	.3335
	50.00	35.2	3.62	7.8	1.009	.1037
	46.88	35.0	.50	1.1	1.000	.0143
	46.38	34.9(Vo)	.00	.0	1.000	.0000
Sample 2.	72.65	46.5	25.89	55.5	1.360	.7570
	69.41	43.9	22.65	48.6	1.284	.6623
	60.46	37.9	13.70	29.3	1.108	.4006
	52.18	35.0	5.42	11.6	1.024	.1585
	47.51	34.5	.75	1.6	1.009	.0219
	47.38	34.5	.62	1.3	1.009	.0181
	46.76	34.2(Vo)	.00	.0	1.000	.0000
Sample 3.	73.09	46.9	25.43	53.5	1.375	.7329
	70.27	44.0	22.61	47.5	1.268	.6516
	62.17	38.1	14.51	30.5	1.098	.4182
	52.89	35.3	5.23	10.9	1.017	.1507
	48.00	34.8	.34	.71	1.026	.0092
	47.98	34.8	.32	.67	1.026	.0092
	47.66	34.7(Vo)	.00	.0	1.000	.0000
Sample 4.	73.30	47.0	25.70	54.0	1.378	.7537
	70.06	43.8	22.46	47.3	1.284	.6584
	61.31	37.7	13.71	28.8	1.106	.4021
	51.17	34.9	3.57	7.5	1.024	.1047
	47.79	34.1	.19	4.0	1.000	.0056
	47.84	34.2	.24	.5	1.000	.0070
	47.60	34.1(Vo)	.00	.0	1.000	.0000

Gros Ventre Non-Sterilized

	Wt. of soil cylinder in grams.	Vol. of soil cyl. in cc's (V)	Wt. of water in grams	% water by wt.	Vol. soil reduced $\frac{(V)}{(Vo)}$	Water lost by 1 cc. oven dry soil $\frac{(\text{wt. water})}{Vo}$
Sample 1.	64.78	36.7	16.5	34.2	1.192	.5357
	62.50	34.0	14.22	29.3	1.104	.4617
	55.49	31.7	7.21	14.9	1.029	.2341
	51.72	31.0	3.44	7.1	1.006	.1117
	49.27	30.8	.99	2.0	1.000	.0321
	48.33	30.8	.00	.0	1.000	.0000
	48.28	30.8(Vo)	.00	.0	1.000	.0000
Sample 2.	65.55	38.0	17.55	36.5	1.218	.5625
	63.07	35.3	15.07	31.4	1.131	.4830
	55.62	32.2	7.62	15.8	1.020	.2442
	51.90	31.9	3.90	8.1	1.022	.1250
	49.18	31.5	1.18	2.5	1.010	.0378
	48.17	31.2	.17	.35	1.000	.0054
	48.15	31.2	.15	.31	1.000	.0048
	48.00	31.2(Vo)	.00	.00	1.000	.0000
Sample 3.	66.72	39.0	18.90	39.8	1.291	.6058
	63.72	36.0	15.90	33.4	1.192	.5096
	55.16	32.0	7.34	15.4	1.026	.2353
	51.51	31.8	3.69	7.24	1.019	.1183
	49.01	31.2	1.19	2.5	1.000	.0382
	48.05	31.2	.23	.48	1.000	.0073
	47.93	31.2	.11	.23	1.000	.0035
	47.82	31.2(Vo)	.00	.0	1.000	.0000
Sample 4.	64.62	37.3	16.44	34.2	1.166	.5137
	62.36	35.0	14.18	29.4	1.094	.4431
	53.51	33.0	5.33	11.0	1.031	.1666
	51.05	32.5	2.87	5.95	1.016	.0897
	49.05	32.2	.87	1.8	1.006	.0271
	48.76	32.0	.58	1.2	1.000	.0181
	48.18	32.0(Vo)	.00	.0	1.000	.0000

Gros Ventre Sterilized

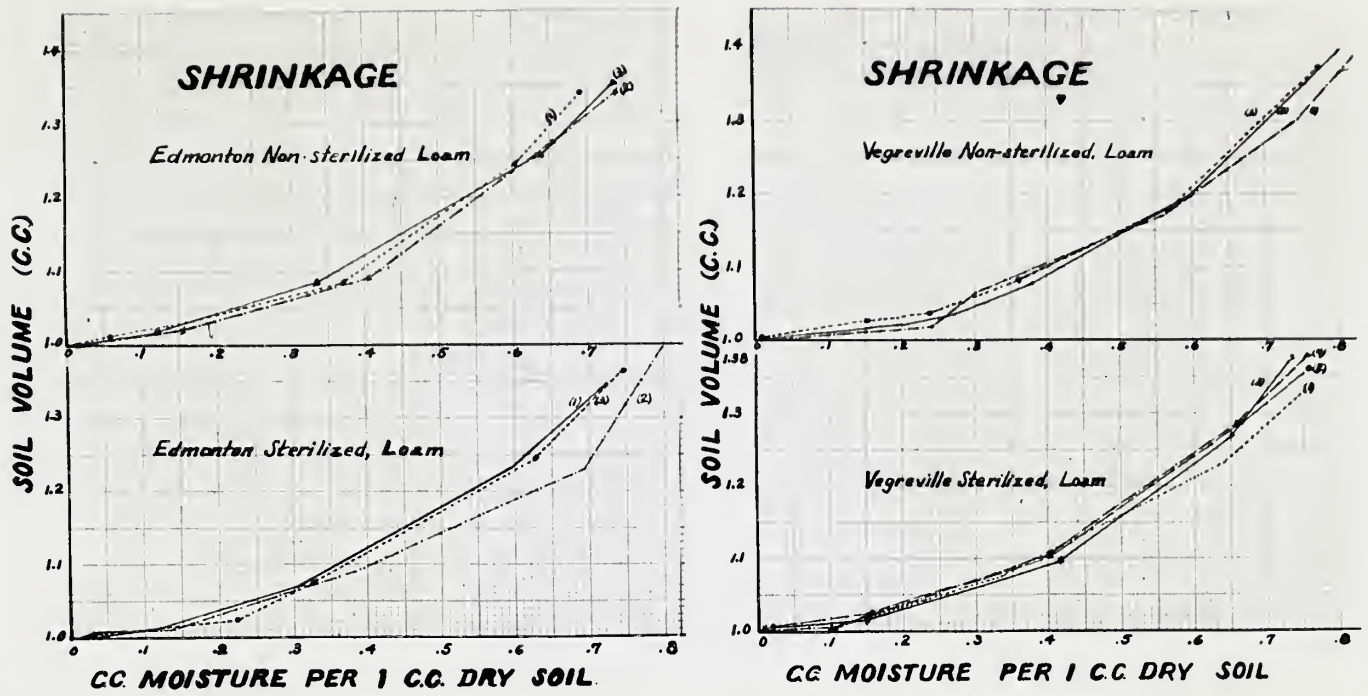
	Wt. of soil cylinder in grams	Vol. of soil cyl. in cc's (V)	Wt. of water in grams	% water by wt.	Vol. soil reduced $\frac{(V)}{(Vo)}$	Water lost by 1 cc. oven dry soil $\frac{(wt.water)}{Vo}$
Sample 1.	65.37	38.2	17.50	36.81	1.225	.5645
	62.94	35.9	15.05	31.50	1.158	.4840
	54.54	32.0	6.67	13.90	1.032	.2152
	49.52	31.2	1.65	3.50	1.006	.0532
	47.96	31.2	.09	.39	1.006	.0290
	47.92	31.2	.05	.10	1.000	.0150
	47.87	31.0(Vo)	.00	.00	1.000	.0000
Sample 2.	67.07	39.9	18.65	37.8	1.247	.5960
	64.63	37.3	16.21	33.2	1.166	.5066
	55.76	33.0	7.34	14.8	1.031	.2294
	50.22	32.0	1.80	3.65	1.000	.0563
	48.50	32.0	.08	.16	1.000	.0280
	48.42	32.0(Vo)	.00	.00	1.000	.0000
Sample 3.	67.71	40.4	19.41	40.3	1.282	.6291
	65.60	37.9	17.30	35.8	1.203	.5492
	56.01	32.7	7.71	16.0	1.038	.2448
	49.92	31.5	1.62	3.4	1.000	.0514
	48.30	31.5(Vo)	.00	.0	1.000	.0000
Sample 4.	65.92	39.1	17.31	35.6	1.253	.5548
	63.52	36.3	14.88	30.6	1.164	.4800
	53.61	32.0	4.97	10.2	1.020	.1593
	49.81	31.3	1.17	2.4	1.003	.0375
	48.67	31.2	.03	.06	1.000	.0009
	48.64	31.2(Vo)	.00	.0	1.000	.0000

Fallis Non-Sterilized

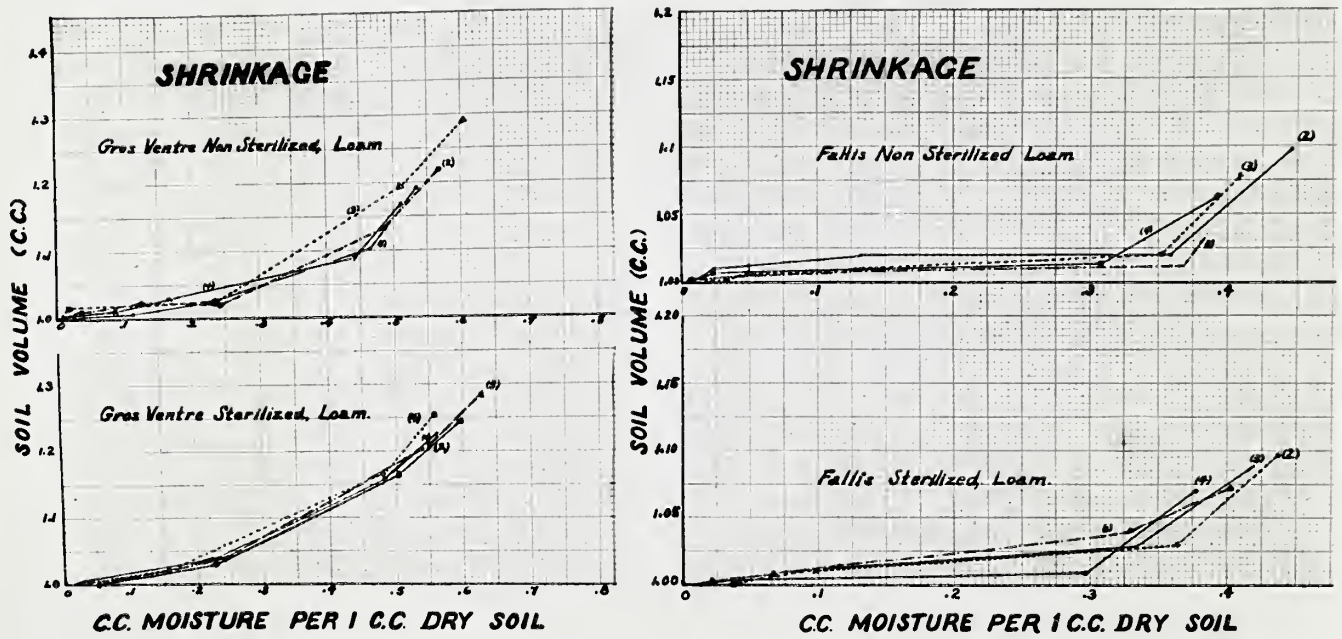
	Wt. of soil cylinder in grams	Vol. of soil cyl. in cc's (V)	Wt. of water in grams	% water by wt.	Vol soil reduced $\frac{(V)}{(Vo)}$	Water lost by 1 cc. oven dry soil $\frac{(wt.water)}{Vo}$
Sample 1.	59.94	30.9	11.47	25.8	1.033	.3836
	57.56	30.3	11.09	22.95	1.013	.3709
	50.30	30.3	1.83	3.78	1.013	.0612
	49.35	30.0	.88	1.87	1.003	.0294
	49.14	30.0	.67	1.38	1.003	.0224
	48.77	30.0	.30	.62	1.003	.0100
	48.64	29.9	.17	.35	1.000	.0057
	48.47	29.9(Vo)	.00	.00	1.000	.0000
Sample 2.	61.24	32.4	13.21	27.4	1.098	.4478
	58.59	30.1	10.56	22.0	1.020	.3580
	51.92	30.0	3.89	8.1	1.020	.1319
	49.48	29.9	1.45	3.0	1.013	.0492
	48.78	29.8	.75	1.56	1.010	.0250
	48.31	29.5	.28	.58	1.000	.0095
	48.27	29.5	.24	.50	1.000	.0081
	48.03	29.5	.00	.00	1.000	.0000
Sample 3.	60.94	32.4	12.15	25.8	1.077	.4091
	58.87	30.3	10.44	21.6	1.020	.3515
	52.12	29.9	3.69	7.6	1.007	.1243
	49.86	29.9	1.43	2.9	1.006	.0481
	49.22	29.7	.99	2.0	1.000	.0333
	48.64	29.7	.21	.43	1.000	.0070
	48.43	29.7	.00	.0	1.000	.0000
Sample 4.	60.39	31.9	11.81	24.3	1.063	.3937
	58.29	30.4	9.71	20.1	1.013	.3057
	50.05	30.4	1.47	3.0	1.006	.0217
	49.23	30.2	.65	1.33	1.003	.0216
	49.27	30.1	.69	1.42	1.003	.0230
	48.74	30.0	.16	.33	1.000	.0053
	48.80	30.0	.22	.45	1.000	.0073
	48.58	30.0	.00	.00	1.000	.0000

Fallis Sterilized

	Wt. of soil cylinder in grams	Vol. of soil cyl. in cc's (v)	Wt. of water in grams	% water by wt.	Vol. soil reduced $\frac{(V)}{(Vo)}$	Water lost by 1 cc. oven dry soil $\frac{(wt.water)}{Vo}$
Sample 1.	60.76	32.0	12.16	25.0	1.070	.4067
	58.57	31.1	9.97	20.5	1.040	.3334
	50.65	30.1	2.05	4.22	1.007	.0685
	49.25	30.0	.65	1.34	1.002	.0219
	49.23	30.0	.63	1.3	1.002	.0211
	48.90	29.9	.30	.62	1.000	.0020
	48.60	29.9	.00	.0	1.000	.0000
Sample 2.	61.95	32.9	13.08	28.3	1.096	.4360
	59.85	31.0	10.98	22.5	1.032	.3660
	51.81	30.3	2.94	6.04	1.010	.0980
	49.94	30.0	1.07	2.19	1.000	.0357
	49.12	30.0	.25	.51	1.000	.0080
	49.06	30.0	.19	.39	1.000	.0063
	48.87	30.0	.00	.0	1.000	.0000
Sample 3.	61.05	32.4	12.41	25.5	1.086	.4151
	58.64	30.7	10.00	20.5	1.027	.3344
	50.91	30.2	2.27	4.65	1.010	.0759
	49.40	30.0	.76	1.56	1.003	.0254
	49.24	30.0	.60	1.26	1.003	.0200
	48.76	29.9	.12	.25	1.002	.0040
	48.64	29.9	.00	.00	1.000	.0000
Sample 4.	60.08	32.0	11.27	23.0	1.071	.3769
	57.71	30.1	8.90	18.2	1.007	.2977
	50.03	30.0	1.22	2.5	1.003	.0408
	49.42	30.0	.61	1.25	1.003	.0204
	48.95	29.9	.14	.29	1.000	.0046
	48.81	29.9	.00	.00	1.000	.0000



Graph 2. Shrinkage of non-sterilized and steam sterilized Edmonton and Vegreville black park soils.



Graph 3. Shrinkage of non-sterilized and steam sterilized Gros Ventre brown prairie soil and Fallis gray wooded soil.

Sticky Point.

The "sticky point" appears to be reached at a lower moisture content in the sterilized soils than in the non-sterilized. The "sticky point" was reached when Edmonton non-sterilized soil was brought to 60 percent moisture content (air-dry basis) and sterilized was brought to 58 percent.

Vegreville, Gros Ventre and Fallis non-sterilized showed the sticky points at 52, 50 and 33 percent respectively, and the sterilized at 48, 46 and 33 percent of moisture.

It was found that at 39 percent and 41.5 percent moisture, Edmonton sterilized was more sticky than the non-sterilized of the same moisture content.

In cultivating the soils in the flasks incubated for nitrification, it was observed that those which were kept sterile for a long time became quite sticky upon addition of water to bring the soils to optimum moisture, while the non-sterilized and the sterilized re-inoculated, with the same moisture content (flasks were all weighed for optimum moisture when the experiment was set up), were not inclined to become sticky.

Capillary Rise.

Table 6 and graphs 4 and 5 show the results of the capillary experiment. Consistent and definite differences were found. In all cases the capillary rise after the first twelve hours was greater in the non-sterilized soils.

Differences became apparent even after 15 minutes in Fallis and Gros Ventre soils and after one hour in Vegreville soil. One sample of Edmonton non-sterilized soil also showed a greater rise after one hour but one of the duplicates was sluggish at the beginning and remained as low as the sterilized up to 24 hours.

The most rapid rise took place in Gros Ventre up to 4 hours but at 12 hours Fallis non-sterilized rose highest of all and remained so till the end.

The highest rise at the end of the experiment was found in Fallis soil for both the non-sterilized and the sterilized treatments. Of the non-sterilized, the next highest rise took place in Edmonton soil, a lower rise in Vegreville soil and the lowest in Gros Ventre. Of the sterilized treatments, the rise was in the same order although in none of them was the rise as high as in the non-sterilized soils.

Capillary movement ceased in 3 days in Gros Ventre and in Vegreville soils that were sterilized, but persisted very slowly even after 2 weeks in the other two soils.

Of the possible explanations for these differences one may say that temperature did not enter as a factor since all the soils were under the same conditions after sterilization. The experiment was conducted at room temperature, which did not vary

very much, so that the differences were not caused by changes in viscosity of water.

Regarding the structure: compacting was uniform and granulation was the same insofar as the soils were all passed through the same sieve. What differences in structure existed were mostly due to the nature of the soils and to those changes induced by sterilization.

Differences in the rise of different soils would be due to texture. For example, Fallis soil is silty in nature and showed the most rapid and the greatest rise, while Edmonton soil was slow at the start because of its more colloidal nature.

It would appear that the lower rise in sterilized may be explained on the basis of soluble organic matter and salts to some extent, the content of these being increased by steam sterilization. Lyon and Bizzell (14) found water soluble matter was increased by steaming. Pickering (12) found total solids in the soil extract increased in steamed soil. But this alone would hardly be sufficient to account for such big differences, as the increases in water soluble matter or total solids are not so very great.

Comparing the capillary rise in sterilized with that in the non-sterilized soils after 58 days, the sterilized treatments of Edmonton, Vegreville, Gros Ventre and Fallis soils showed only 50.7, 38.0, 46.5 and 62.9 percent of the capillary rise in the corresponding non-sterilized soils. That is to say, sterilization decreased capillary rise by 49.3, 62.0, 53.5 and 37.1 percent respectively.

These differences cannot be attributed to initial moisture content, for a moisture determination made on a portion of the

samples used in setting up this experiment showed 4.0, 3.5, 2.5 and 1.6 percent respectively in Edmonton, Vegreville, Gros Ventre and Fallis non-sterilized soils; and 3.8, 3.4, 2.9 and 1.3 percent in the sterilized soils. Therefore, they had the same initial moisture content. Differences in the thickness of the water films, however, may exist due to the tightly held water of hydration of the salts and colloids remaining even after the hygroscopic moisture was driven off. Therefore, differences in the thickness of the water films might account in part for the differences in capillary rise between the sterilized and the non-sterilized soils.

In almost all the work there was a marked difference observed in the color of the soil extracts following sterilization. The sterilized soil extracts appeared darker in color and often colloidal filtrates which were hard to clear up were obtained, indicating that in the capillary experiment colloidal material also probably tended to reduce the rise.

Table 6 - Capillary Rise (in inches) in Sterilized and Non-sterilized Alberta soils.

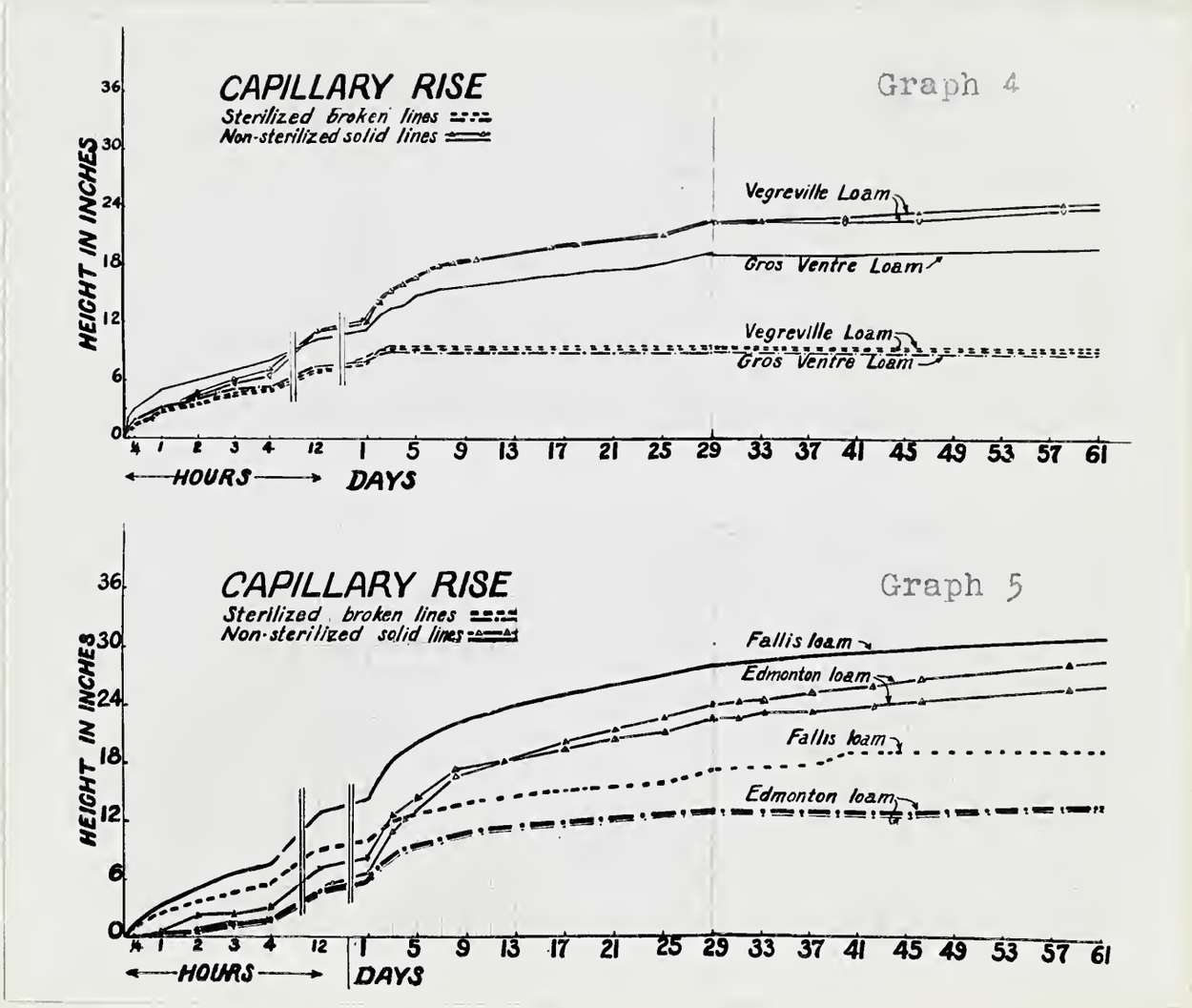
Edmonton Non-ster.	Edmonton Non-ster.	Edmonton Ster.	Edmonton Ster.	Vegreville Non-ster.	Vegreville Non-ster.	Vegreville Ster.	Vegreville Ster.	Gros Ventre Non-ster.	Gros Ventre Ster.	Fallis Non-ster.	Fallis Ster.	Time
0	0	0	0	.8	.5	.8	.8	2.3	1	.5	.5	5 min.
0	0	0	0	1.5	1.3	1.5	1.5	3	1.8	1.5	1	15 "
0	.5	0	0	3.5	3	2.8	3	5	3.3	3.5	2.3	1 hr.
.5	2.3	.8	.5	4.8	4.5	3.5	3.8	6.3	4	5	3.5	2 "
1.3	2.3	1.5	1	6.3	5.8	4.8	4.5	7.3	5	6.5	4.5	3 "
1.5	3	2	1.5	7	6.5	5.3	5.3	8	5.3	7.5	5.3	4 "
5.3	7	5.3	4.5	11	11	7.5	7.8	10.8	7.5	12.8	8.8	12 "
6.5	8.3	6.3	5.5	12	12	8	8.3	11.5	8	14	9.8	24 "
9.3	11	8.3	7.5	14	14.3	8.3	9	12.8	8.8	16.8	11.3	2 days
11	12.5	9	8.5	15.3	15.3	9	9	13.5	9	18.3	12	3 "
12.3	13.5	9.8	9	16	16	9	9	14	9	19.3	12.5	4 "
13.3	14.3	10	9.5	16.5	16.5	9	9	14.5	9	20	12.8	5 "
15.3	16	10.5	10.3	17.5	17.5	9	9	15.3	9	21.8	13.8	7 "
16	16.5	10.8	10.5	18	18	9	9	15.5	9	22	13.8	8 "
16.5	17	11	10.8	18	18.3	9	9	15.5	9	22.5	13.8	9 "
17	17.3	11	11	18.3	18.5	9	9	15.8	9	22.8	14	10 "
17.8	17.8	11.3	11.3	18.5	18.5	9	9	16	9	23.3	14.3	11 "
18	18	11.3	11.3	18.8	18.8	9.3	9	16.3	9	23.5	14.5	12 "
18.8	18.5	11.5	11.5	19.3	19.3	9.3	9	16.5	9	24	14.8	14 "
19.3	18.8	11.5	11.5	19.5	19.5	9.3	9	16.5	9	24.3	14.8	15 "
19.8	19	11.8	11.8	19.8	19.8	9.3	9	16.8	9	24.8	15	16 "
20	19.3	11.8	11.8	20	19.8	9.3	9	17	9	25	15	17 "
20.5	19.5	11.8	11.8	20	20	9.3	9	17	9	25.3	15.3	18 "

Table 6 - Capillary rise (in inches) in saturated and non-saturated Alberta soils.

Table 6 continued - Capillary Rise (in inches) in Sterilized and Non-sterilized Alberta soils.

Edmonton Non-ster.	Edmonton Non-ster.	Edmonton Ster.	Edmonton Ster.	Vegreville Non-ster.	Vegreville Non-ster.	Vegreville Ster.	Vegreville Ster.	Gros Ventre Non-ster.	Gros Ventre Ster.	Fallis Non-ster.	Fallis Ster.	Time
20.8	19.8	11.8	12	20.3	20.3	9.3	9.3	17.3	9.3	25.5	15.3	19 days
21.3	20.3	12	12	20.5	20.5	9.3	9.3	17.3	9.3	25.8	15.5	21 "
21.5	20.3	12	12	20.5	20.5	9.3	9.3	17.5	9.3	26	15.5	22 "
22	20.5	12	12.3	20.8	20.8	9.3	9.3	17.5	9.3	26.3	15.5	23 "
22.3	20.8	12	12.3	20.8	21	9.3	9.3	17.8	9.3	26.5	15.8	24 "
22.5	21	12.3	12.3	21	21	9.3	9.3	17.8	9.3	26.8	15.8	25 "
22.8	21.3	12.3	12.3	21.3	21.3	9.3	9.3	18	9.3	27	16	26 "
23.5	22	12.5	12.5	22	22	9.3	9.3	18.5	9.3	27.5	16.5	28 "
23.8	22.3	13	13	22.3	22.3	9.3	9.3	18.8	9.3	28	17.3	29 "
23.8	22.3	13	13.3	22.3	22.3	9.3	9.3	19	9.3	28	17.3	30 "
24	22.3	13	13.3	22.3	22.3	9.3	9.3	19	9.3	28.3	17.3	31 "
24.3	22.5	13	13.3	22.5	22.3	9.3	9.3	19	9.3	28.5	17.3	32 "
24.5	22.8	13	13.3	22.5	22.5	9.3	9.3	19	9.3	28.8	17.3	33 "
25	23	13	13.3	22.5	22.5	9.3	9.3	19	9.3	28.8	17.3	35 "
25	23	13	13.3	22.8	22.5	9.3	9.3	19	9.3	29	17.3	36 "
25.3	23	13	13.3	22.8	22.5	9.3	9.3	19	9.3	29	17.5	37 "
25.3	23.3	13	13.3	22.8	22.5	9.3	9.3	19	9.3	29	18	38 "
25.3	23.3	13	13.3	22.8	22.5	9.3	9.3	19	9.3	29.3	18.3	39 "
25.5	23.5	13	13.3	23	23	9.3	9.3	19	9.3	29.3	19.3	40 "
26	24	13	13.3	23.3	23	9.3	9.3	19.3	9.3	29.5	19.5	42 "
26.8	24.5	13	13.3	23.5	23.3	9.3	9.3	19.5	9.3	30	19.5	46 "
28.5	25.8	13.5	14	24.5	24	9.3	9.3	20	9.3	31	19.5	58 "
28.5	25.8	13.5	14	24.8	24.3	9.3	9.3	20	9.3	31.3	19.5	61 "

Table 6 continued - Cervical size (in tonnes) in thrashing and non-threshing Alvarka maize.



Graphs 4 & 5. Capillary rise in non-sterilized and steam sterilized Edmonton and Vegreville black park soils, Gros Ventre brown prairie soil and Fallis gray wooded soil.

CHEMICAL-BIOLOGICAL.

pH. Values.

Schreiner and Lathrop (13) in 1912 found an increase in total soil acidity when they steam sterilized the soil for 3 hours at 135 degrees C. They probably did not determine the hydrogen ion concentration but titrable acidity.

In this investigation, as shown by table 7, no significant differences were found in pH values between sterilized and non-sterilized as measured by the quinhydrone electrode, when the soils were steam sterilized twice, on successive days, once for three quarters of an hour and the second time for half an hour at 120 degrees C.

Table 7 - Hydrogen ion concentration of four sterilized and non-sterilized Alberta soils, expressed as pH.
(Determined by the quinhydrone electrode)

	Sterilized				Non-sterilized			
	1 min.	5 min.	15 min.	30 min.	1 min.	5 min.	15 min.	30 min.
Edmonton	6.0	6.1	6.1	6.0	6.3	6.1	6.2	6.0
black	6.1	6.1	6.1	6.1	6.3	6.1	6.3	6.0
park	6.0	6.1	6.1	6.1	6.2	6.2	6.1	6.2
Average	6.0	6.1	6.1	6.1	6.3	6.1	6.2	6.1
Vegreville	6.3	6.1	6.1	6.2	6.2	6.2	6.1	6.2
black	6.2	6.1	6.1	6.2	6.2	6.1	6.0	6.2
park	6.2	6.1	6.1	6.2	6.3	6.1	6.2	6.1
Average	6.2	6.1	6.1	6.2	6.2	6.1	6.1	6.2
Gros Ventre	5.8	5.7	5.8	5.8	6.0	5.8	5.9	5.9
brown	5.8	5.8	5.8	5.7	5.9	5.8	5.9	5.9
prairie	5.7	5.7	5.8	5.7	5.8	5.8	5.9	5.9
Average	5.8	5.7	5.8	5.7	5.9	5.8	5.9	5.9
Fallis	6.1	6.2	6.2	6.2	6.3	6.1	6.2	6.1
gray	6.1	6.2	6.2	6.2	6.3	6.1	6.2	6.2
wooded	6.1	6.2	6.2	6.2	6.3	6.1	6.2	6.2
Average	6.1	6.2	6.2	6.2	6.3	6.1	6.2	6.2

Water Soluble Phosphorus.

Water soluble phosphorus in sterilized soils is increased as shown in table 8. By comparison with the non-sterilized soils the amounts were found to be 2.8 times in the Edmonton black soil, 1.7 times in Vegreville soil, 2.1 times in Gros Ventre brown prairie soil, and a slight increase in Fallis gray wooded soil.

Without ignition of organic matter dissolved in the water extracts, the amounts of water-soluble phosphorus were found to be approximately half as great.

The change induced by sterilization in water soluble phosphorus content was large in the soils high in organic matter, and relatively small in the Fallis soil, suggesting that organic phosphorus compounds are partly broken down or in some way made more soluble and are later broken down by ignition.

Schreiner and Lathrop (13) found that steam sterilization affected solubility of soil constituents: "By heating there was an increase in all of the constituents isolated from the unheated soil except nucleic acid", and "By the process of heating there was formed xanthine, hypoxanthine, guanine, cytosine and arginine when not previously existing. These products are decomposition products of nucleic acid and protein material and are all beneficial to plant growth."

Table 8 - Water soluble Phosphorus in non-sterilized and sterilized Alberta soils.

(Average p.p.m.P. on water free basis)

Soil	Non-sterilized p.p.m. P.	No. of determinations	Ave. deviation	Sterilized p.p.m. P.	No. of determinations	Ave. deviation.
Edmonton black park	1.67	12	.47	4.69	18	.48
Vegreville black park	4.17	5	.22	7.01	5	.2
Gros Ventre brown prairie	3.16	4	.05	6.72	4	.05
Fallis gray wooded	1.38	4	.01	1.83	4	.04
		Without Ignition.				
Vegreville black park	2.25	4	.37	3.8	4	.7
Gros Ventre brown prairie	1.0	4	.05			

Easily Soluble Phosphorus.

Table 9 shows the quantities of easily soluble phosphorus in the non-sterilized, sterilized and sterilized re-inoculated (with original soil) four Alberta soils, throughout the incubation period of 20 weeks following sterilization.

In general, the trend, especially in the black Edmonton and Vegreville soils, seems to be that greater quantities of easily soluble phosphorus are found in the sterilized and sterilized re-inoculated than in the non-sterilizaed soils, but the increases are very small. While close agreement was obtained in reading the duplicates of a single determination, a variation of 10 parts per million of phosphorus was often found on separate determinations.

However, taking the averages for each treatment of each soil, over the whole period of incubation, and comparing the averages thus obtained, it appears that sterilization and incubation have increased the content of easily soluble phosphorus.

The increases of sterilized over non-sterilized found in each soil, comparing the final averages (the figures in the last right hand column of table 9, worked out on a percentage basis) were 37.2 percent in the case of Edmonton, 12.5 percent in Vegreville and 7.9 percent in Fallis soils. No significant difference was found in Gros Ventre soil though the sterilized was approximately 2 percent higher. The sterilized re-inoculated were still higher than the sterilized alone but only by 4.8 percent, 4.5 percent and 3.4 percent in Edmonton, Vegreville and Gros Ventre soils.

The increase with time of incubation might be attributed to the greater microbiological activity in the non-sterilized and sterilized re-inoculated soils, following re-moistening and cultivation, resulting in a greater production of acids including carbon-dioxide from the activity of the micro-organisms, which would form carbonic acid and act on the inorganic as well as the organic phosphorus compounds of the soil thereby bringing more easily soluble phosphorus into solution. The micro-organisms very likely utilize certain phospho-proteins and cause an accumulation of phosphorus as a by-product. But the sterilized soils also increased in easily soluble phosphorus. The increases here cannot be attributed to microbial activity, unless all soils became contaminated following sterilization. It is possible that some flasks were contaminated since they were opened to be watered once every week or ten days, but asceptic technique was used, so any contaminations would be purely accidental and it is unlikely that all the flasks were contaminated. Besides, increases in the sterilized treatments were found even right after sterilization so that these must be attributed to the changes brought about by sterilization. Heating would tend to hasten the decomposition of the soil in the same way as weathering over a long period of time. It would tend to decompose some organic and possibly inorganic phosphorus compounds of the soil. Heating combined with moistening would tend to dissolve the phosphorus contained in the inner structure of the soil particles and drying might crystallize it on the outer shell of the soil particles, thereby increasing the amount of

easily soluble phosphorus.

Baldwin (23) found more easily soluble phosphorus in sterilized soil in the greenhouse. Darbishire and Russell (34) found that plants grown on sterilized soil utilized about twice as much phosphorus as those plants grown on normal soil, and that the percentage of phosphorus in the plant grown on sterilized soil was much higher than that on the normal soil.

The first column in table 9 shows the easily soluble phosphorus in the non-sterilized and sterilized four Alberta soils, right after sterilization. These figures were obtained on samples brought into the laboratory in the fall of 1936. The rest of the figures in table 9 were obtained from the samples incubated for ammonia, which experiment was set up in the fall of 1937 when a fresh supply of soil was brought in. The figures in the first column are the averages of several different determinations. That is to say, the non-sterilized, Edmonton, Vegreville, Gros Ventre and Fallis figures are averages of 15, 6, 6 and 5 separate determinations, in which the average deviations were 1.1, 2.0, 3.5, 1.6 p.p.m., and the sterilized are averages of 15, 6, 6, 3 determinations, with an average deviation of 2.1, 2.1, 1.5, 1.5 p.p.m. respectively.

Increases in the sterilized over those in the non-sterilized in easily soluble phosphorus were found to be 22, 31, 11 and 13 percent respectively in the Edmonton, Vegreville, Gros Ventre and Fallis soils.

Table 9 - Easily soluble phosphorus (soluble at pH 3) in non-sterilized, sterilized, and reinoculated sterilized Alberta soils.

(Average p.p.m. P on water-free basis)

			Period of incubation after sterilizing soil and setting up experiment.																					
			3 days		6 days		2 weeks		4 weeks		8 weeks		12 weeks		16 weeks		20 weeks		Fin	
			Dup*	Ave.	Dup.	Ave.	Dup.	Ave.	Dup.	Ave.	Dup.	Ave.	Dup.	Ave.	Dup.	Ave.	Dup.	Ave.	Av	
Edmonton black park	Non-ster.	33			32		35.2		35		46		51		48		54.4			
	Ster.and reinoc.				32	32	28.8	32	40	37	46	46	53	52	50	49	56.0	55	43	
					39		40		56		51		72		72		79.0			
					41	40	48	47	58	57	53	52	76	74	77	75	81.6	80.	61	
	Ster.	40			41		48		56		58		76.8		70.4		62.4			
					43	42	55	51	56	56	54	56	76.8	77	69	70	63.2	63	59	
Vegreville black park	Non-ster.	46			86		86		68		96		89.6		86.4		109.0			
	Ster.and reinoc.				78	82	86	86	70	69	96	96	92.8	91	86.4	86	107.0	108	88	
					78		91		92.8		128		123		102.4		114			
					78	78	87	89	92.8	93	123	125	123	123	104	103	112	113	103	
	Ster.	60			81		98		82.4		104		107		104		112			
					84	83	99	99	83.2	83	100	103	112	109	104	104	109	110	99	
Gros Ventre brown prairie	Non-ster.	56	53.8		48		45.6		56		52		101		55		54.4			
	Ster.and reinoc.		43.2	48	48	48	45.6	46	55.2	56	56	54	96	98	56	56	59.2	57	58	
			42.2		49.2		41		53		59		107		59.2		67.2			
			53.6	48	48.8	49	45	43	53	53	62	61	107	107	62.4	61	67.2	67	61	
	Ster.	62	56		54		47		51		54		80		51.2		62.4			
			56	56	54	54	48	48	53	52	56	55	83	82	54.4	53	59.2	61	59	
Fallis gray wooded	Non-ster.	87	78.4		83		81		91		99		115		75		97.6			
	Ster.and reinoc.		75.2	76	83	83	83	82	92	92	101	100	115	115	77	76	102.4	100	90	
			83.2		86		80		104		102		120		89.6		102.4			
			88	85	86	86	83	82	102	103	102	102	120	120	92	91	107.0	105	97	
	Ster.	99	88		91		80		93		110		127		83.2		99.2			
			91	90	91	91	83	82	94	94	102	106	123	125	85	84	102.4	101	97	

*Dup. used to designate the word "duplicates."

Nitrification.

The results of the two nitrification experiments are shown together in table 10 and in graphs 6, 7, 8 and 9.

Greatest nitrification occurred in Vegreville and Edmonton soils, less in Gros Ventre, and least of all in the Fallis soil; that is to say the higher the organic matter content of the soil, the greater the nitrification.

In all cases the sterilized treatments remained very low in nitrates even after 39 weeks of incubation. Edmonton soil in this treatment varied from about 5 to 3 p.p.m.; Vegreville 49 to 39 p.p.m.; Gros Ventre 9 to 2 p.p.m. and Fallis 5 to 1 p.p.m., with a slight decline towards the end of the incubation period.

There was no evidence that nitrates disappear from the soil to any great extent due to sterilization. The first column in table 10 shows that in Gros Ventre and Fallis soil, nitrate content was the same before as after sterilization, while Vegreville soil, which had a high content of nitrate to start with, did show a small decrease of 5 or 6 p.p.m. of nitrate nitrogen. If nitrates disappear to a slight extent it probably is due to the heat effect causing nitrate to evaporate or change to a form which would not be measured by the phenol-di-sulfonic method.

All of the non-sterilized soils gradually accumulated nitrates. The most rapid change took place up to 20 weeks. At the end of 39 weeks, Edmonton non-sterilized contained 294 p.p.m. of nitrate nitrogen, Vegreville 239 p.p.m., Gros Ventre 219 p.p.m. and Fallis soil the least, only 85 p.p.m. These were all lower than the corresponding sterilized re-inoculated after approximately

10 weeks but higher in all cases up to 10 weeks incubation.

The re-inoculated treatments after about 10 weeks showed a much greater nitrate accumulation than the non-sterilized. Edmonton sterilized and re-inoculated exceeded the non-sterilized by 133 p.p.m., Vegreville by 231 p.p.m., Gros Ventre by 39 p.p.m. and Fallis soil by 2 p.p.m. of nitrate nitrogen after 39 weeks.

This distinct change at 10 weeks is best seen by referring to graphs 6, 7, 8 and 9 and seems significant since a reverse change took place in ammonification, although slightly earlier. Either the nitrifying organisms (Nitrosomonas and Nitrobacter) take a long time to become active when re-inoculated into sterilized soil, or sterilization has in some way altered the chemical composition of the soil making it unfavorable for their development until other micro-organisms have time to overcome these inhibiting effects. Once the nitrifiers become established they are able to convert large quantities of ammonia to nitrate and withstand great concentrations of their own product (in spite of the tendency to increase in acidity due to nitric ions). For example, in the Edmonton and Vegreville sterilized re-inoculated soils there was an accumulation of 427 and 470 p.p.m. of nitrate, yet these organisms seem to carry on their work. In the non-sterilized soil, bacteria, fungi, Actinomycetes and to some extent protozoa, are active, feeding on organic matter or its products to obtain food and energy. Proteins, amines and other nitrogenous substances from the organic matter are converted into simpler nitrogen compounds including ammonia and some free nitrogen by a large number of bacteria and fungi, and to a slight extent by

Actinomycetes of the soil. After this first ammonification stage the nitrifying bacteria oxidize the ammonium compounds to nitrous acid and nitrates. As a result the nitrates accumulate in the non-sterilized soil.

In the sterilized soil this biochemical process of breaking down organic matter and converting the ammonia or the ammonium compounds into nitrates has ceased with the destruction of the micro-organisms during sterilization, so that the nitrates remain at the same or even slightly lower level than at the beginning.

In the sterilized soil re-inoculated with the original soil, presumably the same micro-organisms that were present in the non-sterilized soils are active though in small numbers at first. Schreiner and Lathrop (13) found an increase in a large number of nitrogenous compounds following sterilization so that if the micro-organisms in the sterilized re-inoculated soils were as active as in the non-sterilized there should have been greater nitrate accumulation. Table 10 shows that nitrates were depressed until 10 weeks so that we must conclude that sterilization has made the soil, for a time, more toxic to the nitrifying organisms but more favorable afterwards, or else the nitrifying organisms develop slowly in re-inoculated soil as compared to ammonifying organisms.

Table 10 - Nitrification in non-sterilized, sterilized and re-inoculated sterilized Alberta soils.

(Average p.p.m. nitrate-nitrogen on water-free basis)

		Period of incubation after sterilizing soils and setting up experiment								
		Right after ster.	1 day	6 days	2 weeks	4 weeks	6 weeks	8 weeks	10 weeks	12 weeks
Edmonton black park	Non-sterilized	3	6	83	6	32	68	87	95	130
	Sterilized and re-inoculated	3		5	4	8	8	16	56	285
	Sterilized	3	5	19	4	10	5	3	3	3
Vegreville black park	Non-sterilized	44	55	56	56	99	113	133	127	172
	Sterilized and re-inoculated	45	50	42	50	54	59	129	292	340
	Sterilized	48	50	48	43	56	52	47	51	51
Gros Ventre brown prairie	Non-sterilized	6	8	19	3	20	33	44	49	64
	Sterilized and re-inoculated	6		11	3	12	5	7	20	67
	Sterilized	6	9	11	3	11	4	3	4	3
Fallis gray wooded	Non-sterilized	4	4	6	17	67	94	104	107	135
	Sterilized and re-inoculated	3		4	7	11	8	16	60	114
	Sterilized	4	5	5	6	9	13	5	5	6

Table 10 continued - Nitrification in non-sterilized, sterilized and re-inoculated sterilized Alberta soils.

(Average p.p.m. nitrate-nitrogen on water-free basis)

		Period of incubation after sterilizing soils and setting up experiment.							
		12 weeks	14 weeks	16 weeks	18 weeks	20 weeks	26 weeks	30 weeks	39 weeks
Edmonton black park	Non-sterilized	195	213	221	229	271	260	239	294
	Sterilized and re-inoculated	294	353	356	356	400	400	400	427
	Sterilized	4	3	5	5	6	3	1	2
Vegreville black park	Non-sterilized	182	195	182	222	244	222	226	239
	Sterilized and re-inoculated	356	432	458	421	450	468	438	470
	Sterilized	44	20	44	5	47	45	43	39
Gros Ventre brown prairie	Non-sterilized	77	157	145	190	216	200	190	219
	Sterilized and re-inoculated	190	242	193	235	258	235	376	258
	Sterilized	7	9	8	10	10	6	4	1
Fallis gray wooded	Non-sterilized	77	82	69	83	95	77	73	85
	Sterilized and re-inoculated	85	98	91	95	119	100	95	87
	Sterilized	5	3	3	5	6	3	2	2

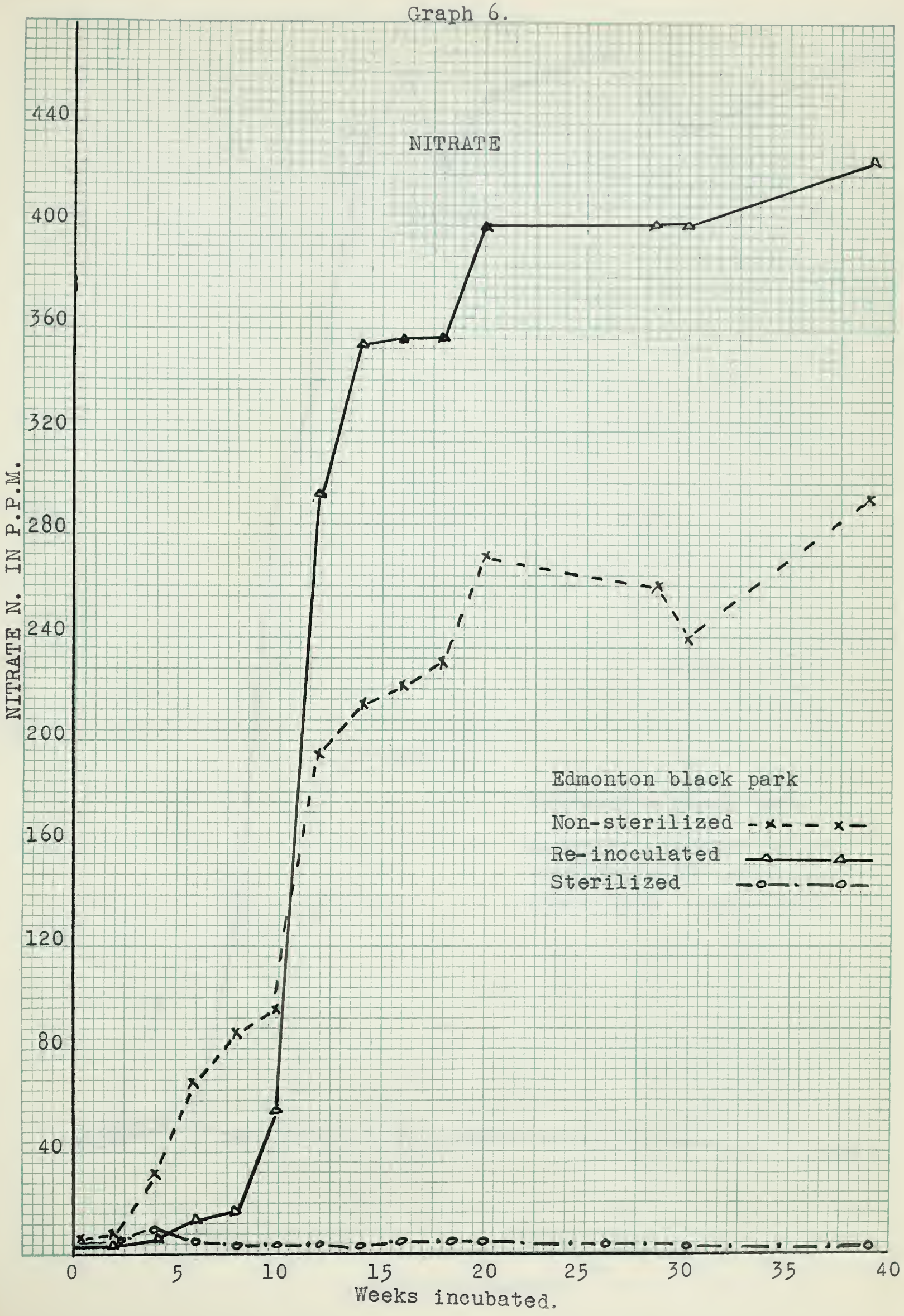
Table 10 continued - Nitrifying power in non-sterilized, sterilized and re-inoculated sterilized Alegre soils.

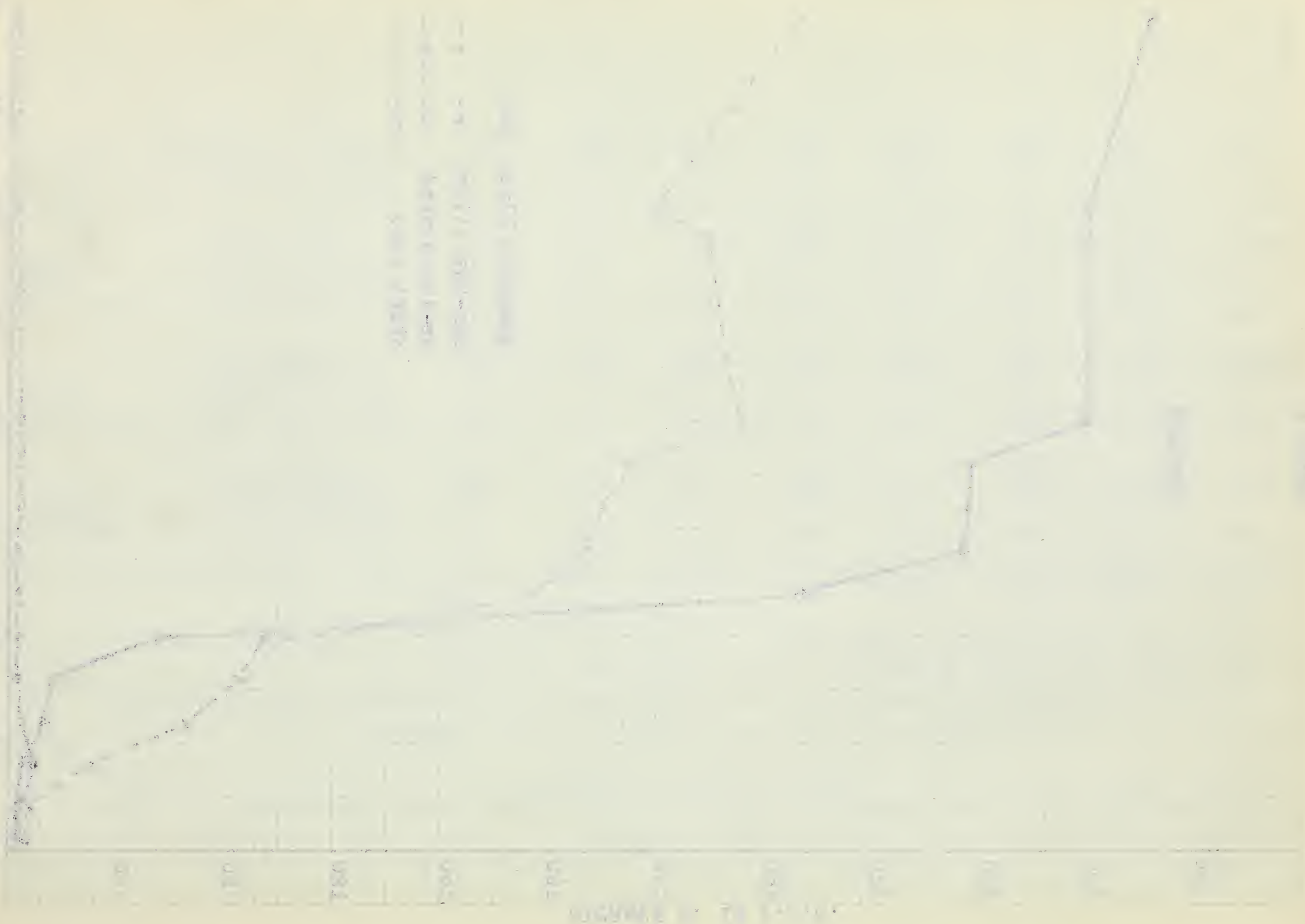
(Average 7 x 3. Nitrate-nitrogen in mgms.-per cent)

Period of incubation after nitrifying soils had reached a maximum.

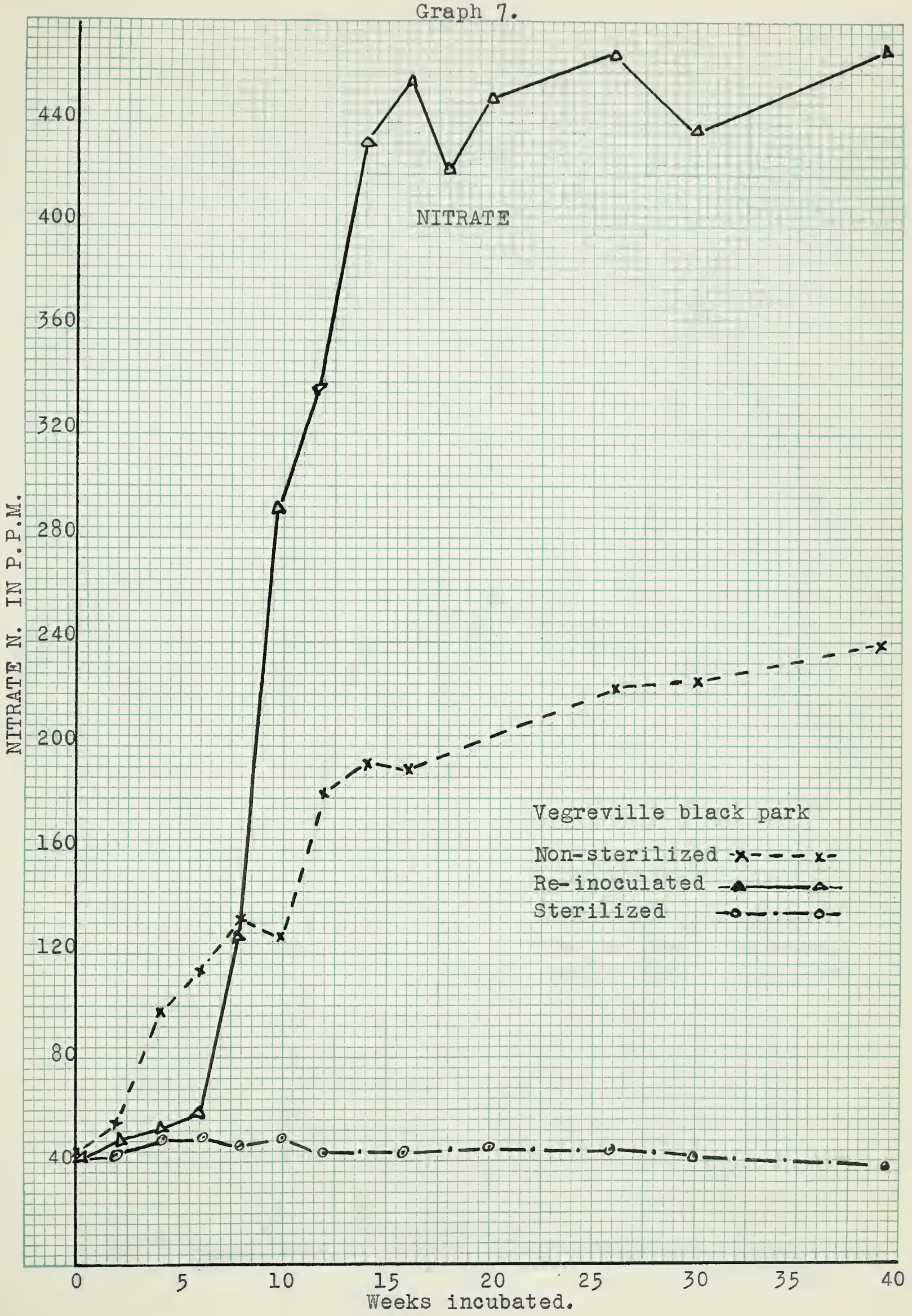
Treatment	1d Weeks	2d Weeks	3d Weeks	4d Weeks	5d Weeks	6d Weeks	7d Weeks
None	Non-sterilized soil	139	219	281	284	274	216	139
	Sterilized soil	25	39	35	175	400	460	427
	Re-inoculated	4	3	3	3	3	3	3
Toluene 1:10	Non-sterilized soil	106	409	1.89	232	244	222	170	139
0.2%	Sterilized soil	4	3	3	3	3	3	3	3
Xylene	Non-sterilized	136	459	486	427	430	162	403	476
0.2%	Sterilized soil	46	24	44	3	47	43	3	39
Iron Sulphate	Non-sterilized	77	137	149	196	224	206	186	159
0.2%	Sterilized soil	3	3	3	3	3	3	3	3
Sulphur	Non-sterilized	140	84	149	126	161	213	178	139
0.2%	Sterilized soil	7	7	8	12	10	8	8	7
Control	Non-sterilized	77	62	59	49	67	77	74	66
None	Sterilized soil	3	3	3	3	3	3	3	3
Control	Re-inoculated	59	66	71	72	114	100	71	67
None	Sterilized soil	3	3	3	3	3	3	3	3

Graph 6.


NITRATE
NITRATE N. IN P.P.M.
440
400
360
320
280
240
200
160
120
80
40
0
5
10
15
20
25
30
35
40
Weeks incubated.
Edmonton black park
Non-sterilized
Re-inoculated
Sterilized



Graph 7.


NITRATE
NITRATE N. IN P.P.M.
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25
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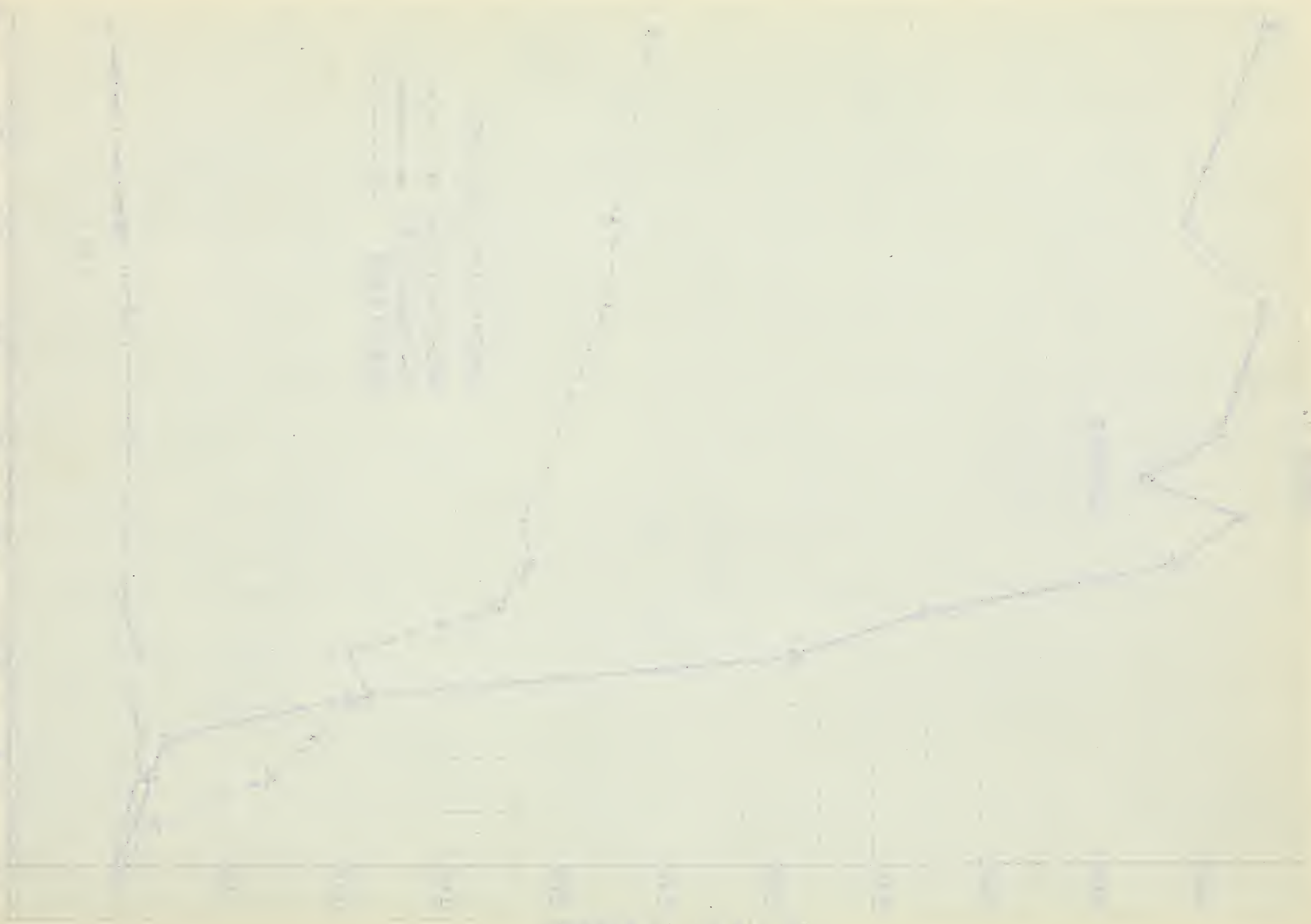
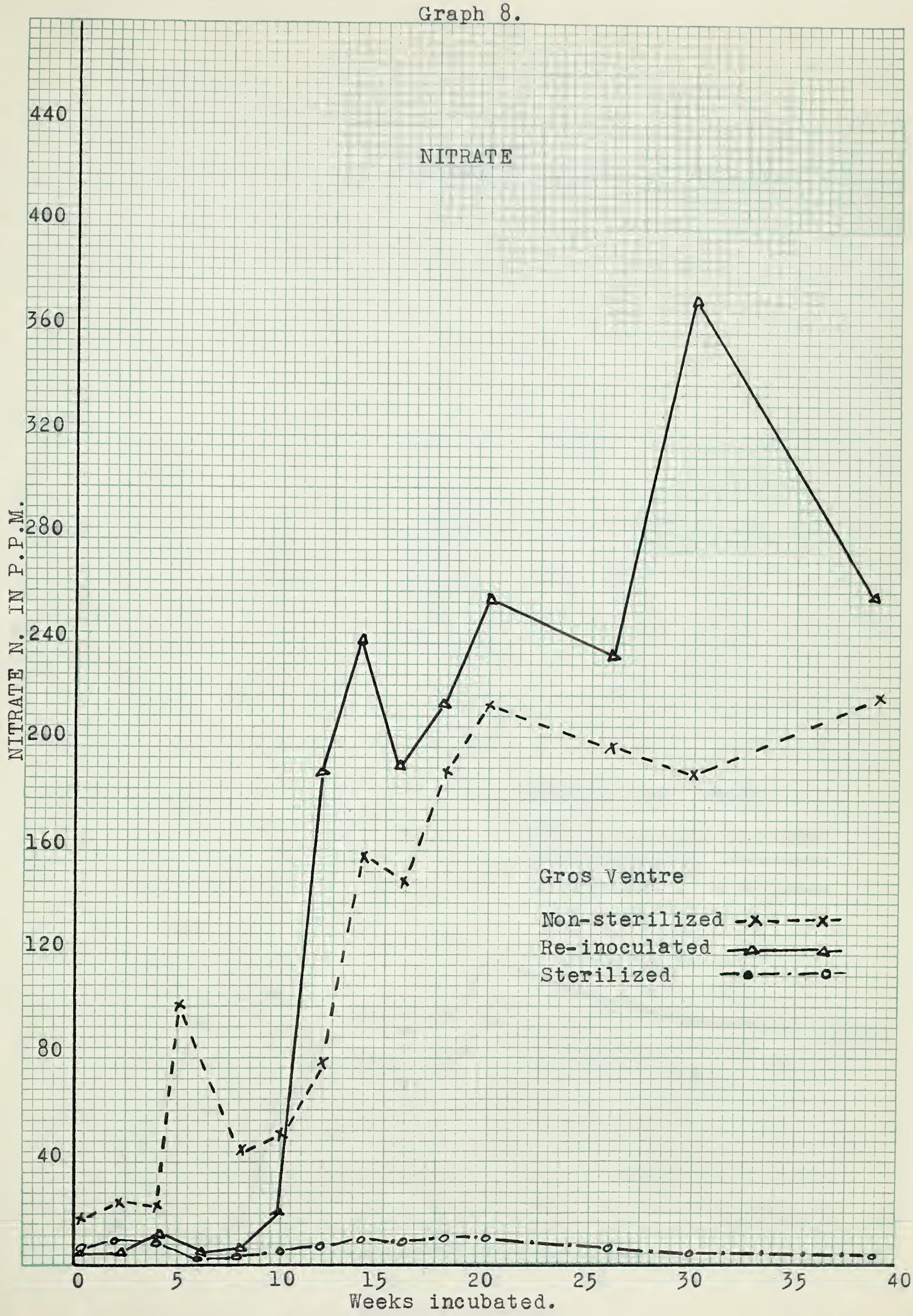
35
40
Weeks incubated.
Vegreville black park
Non-sterilized
Re-inoculated
Sterilized



Graph 8.


NITRATE
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NITRATE N. IN P.P.M.
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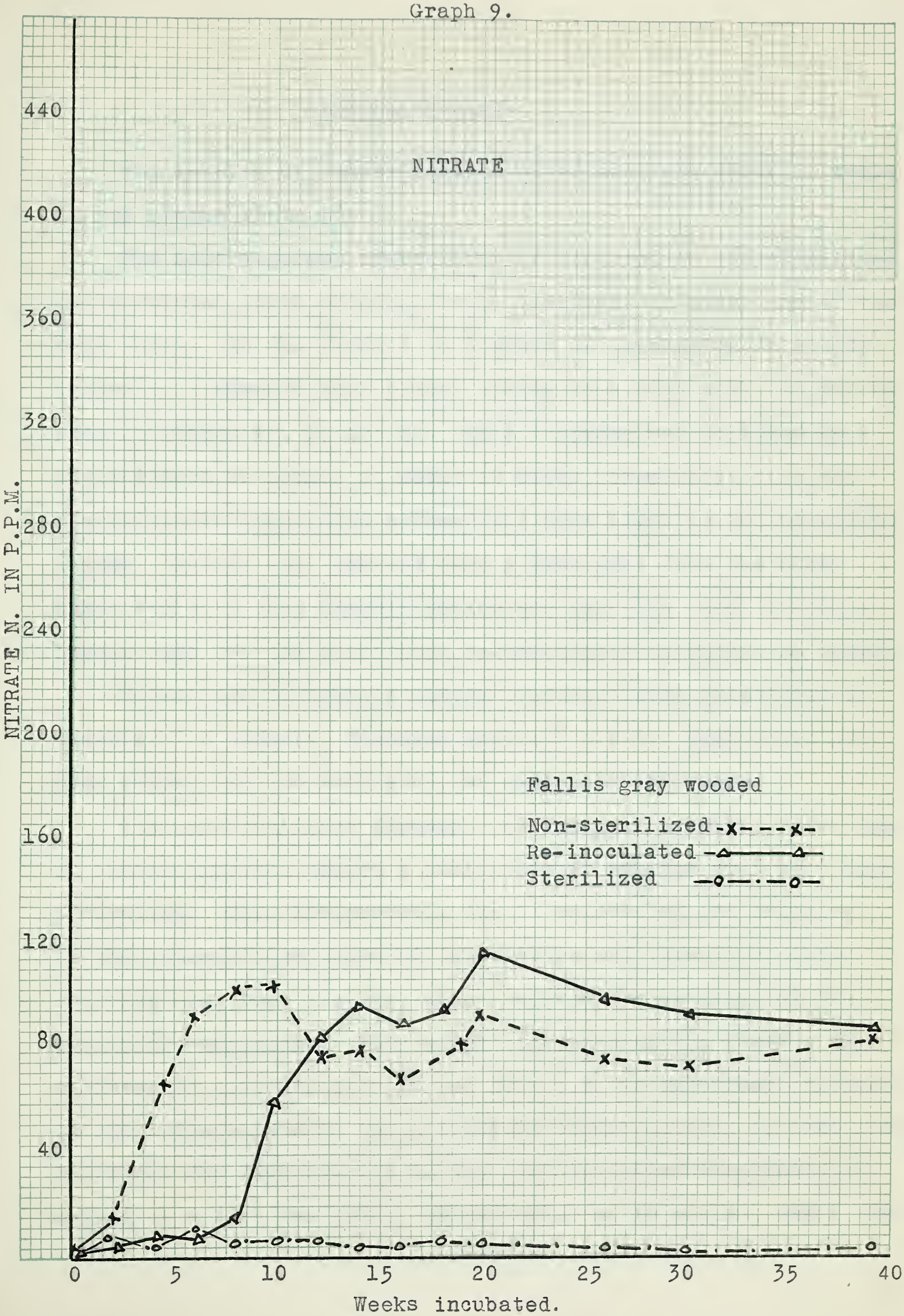
10
15
20
25
30
35
40
Weeks incubated.
Gros Ventre
Non-sterilized
Re-inoculated
Sterilized

Graph 9.


NITRATE
NITRATE N. IN P.P.M.
440
400
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25
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35
40
Weeks incubated.
Fallis gray wooded
Non-sterilized
Re-inoculated
Sterilized

Ammonification.

The results of ammonification experiment are shown in table 11 and graphs 10 to 13.

The most vigorous ammonification occurred in the sterilized re-inoculated (with original soil) treatment in every one of the four soils. In the first 4 to 6 weeks of incubation, a steady increase in ammonia took place, rising to a high peak, as may be seen in graphs 10, 11 and 12. This increase was followed by a rapid return to normal at about 12 weeks, from which time the sterilized re-inoculated assumed the same level as the non-sterilized. Fallis soil behaved very differently from the other three soils in that ammonification was very low, and it took a longer time for the sterilized re-inoculated to return to normal.

Soils high in organic matter produced large quantities of ammonia; for example, Edmonton soil 198 p.p.m., Vegreville 172, Gros Ventre 126 and Fallis 63, of ammoniacal nitrogen.

The non-sterilized Edmonton and Vegreville soils, both high in organic matter, showed a small rise in ammonia on 3 to 6 days incubation. This was probably due to moistening and bringing to the optimum conditions of temperature and structure, soils that had been kept air-dry, which renewed the activity of the soil micro-organisms. This slight increase in the first 6 days amounted to approximately 30 and 50 p.p.m. of ammoniacal nitrogen. It soon disappeared as the nitrifiers became active, so that after the first few days ammonia in the non-sterilized treatments remained at a very low level, in the order of 10 p.p.m., until the

end of the experiment.

The sterilized soils remained at a fairly constant level, below the re-inoculated but above the non-sterilized. Edmonton sterilized remained at about 80 p.p.m., with one fluctuation when it rose to 125 p.p.m., graph 10; the Vegreville sterilized generally remained slightly lower with two fluctuations up to 85 and 96 p.p.m., graph 11; Gros Ventre about 65 p.p.m., graph 12;, and Fallis soil approximately 35 p.p.m. of ammonia, graph 13.

The larger fluctuations in the sterilized soils, as well as high readings, were probably due to contamination from the air. Although great care was exercised when watering the flasks and precautions were taken to avoid re-infection by using sterile water and sterile pipettes and flaming each flask when the plug was removed, still air contamination could have taken place since each flask was opened every week or ten days and kept opened long enough to add the water from a pipette. Besides that, the cotton plugs soon became loose from cultivating the soil by means of the stirring rod imbedded in each plug.

That sterilization was properly carried out was proved by plating out soils sterilized in flasks as for the experiments with nitrates and ammonia, except that the time of sterilization was reduced to 15 minutes at full pressure. No growth in sterilized soils took place when plated out, using Thornton and Gray's medium. This was repeated and confirmed.

A shorter experiment on ammonification was also set up as a duplication of the longer experiment, and ammonia was determined

after 3 and 6 days, 2 weeks and 4 weeks after sterilization. The results of this experiment are not shown in table 11 because the figures show exactly the same trends as those in the first experiment. Much the same quantities of ammonia were found except that Fallis re-inoculated contained a little more at 4 weeks than that shown in table 11 (71 p.p.m. compared to 52 p.p.m. as indicated).

As a comparison with ammonia in the soil under natural conditions, samples of Edmonton soil were brought into the laboratory on October 3, 1937, and ammonia determined at once. Moisture was determined on a separate sample of the same soil. It was found that on that day in the field, Edmonton sod contained 19.5 percent moisture and an average of 8.4 p.p.m. of ammoniacal nitrogen. This, of course, was only a single day's determination, and considerable fluctuation may occur.

Since the method used to determine ammonia involved the use of magnesium oxide to displace the ammonia on distillation, it was thought advisable to find if magnesium oxide would break down dissolved organic matter and liberate ammonia in stages rather than causing it to be given off completely in one stage. On second distillation it was found that a very small amount of ammonia could be obtained from the second distillation. An average of 30 determinations indicates 4 p.p.m. of ammoniacal nitrogen were given off when the second distillation was prolonged for 45 minutes. This indicates that the values obtained in the ammonification experiment are not absolutely quantitative. But since all the distillations were carried out under uniform conditions of distilling for three quarters of an hour from the first boiling, during which time approximately 250 cc. of dis-

tillate were collected, practically all of the ammonia that entered into base exchange or ammonium salts was collected from the first distillation.

The Rate at which Ammonia was Distilled.

It was also found that most of the ammonia was distilled over in 15 minutes from the first boiling. In 4 minutes, more than .56 mgms. of ammoniacal nitrogen were distilled. In 7 minutes, 10, 15, 18 and 20 minutes, more than 1.12 mgms., 2.24, 3.36, 3.92 and 4.48 mgms. of ammoniacal nitrogen were distilled over and collected in standard acid. These figures, expressed on the basis of a 25 gram sample as used in most determinations of ammonia (except where the contents were high when a 12.5 gram sample was used instead), would mean that more than 90 p.p.m. of ammonia in 10 minutes, 135 p.p.m. in 15 minutes and 180 p.p.m. in 20 minutes, were distilled over in the first distillation. Therefore, distilling for 45 minutes from the first boil, i.e., one hour from the time the flames were lighted, would allow well over the time required for complete distillation because the highest amount obtained from any soil in the experiment (see table 11) was less than 200 p.p.m.

Ammonification, like nitrification, is a biochemical process, but unlike the latter it can be carried on by a large number of bacteria (Bacillus mycoides, B. mesentericus vulgatus, etc.) and fungi (as Mucor, Penicillium, Rhizopus, Trichoderma, etc.). Organic matter is decomposed by bacteria and fungi and converted to simpler nitrogen compounds and ammonia.

Sterilization partly decomposes organic matter (13) which is more easily attacked than undecomposed organic matter.

The maximum rise in ammonia took place in about 4 weeks, after that it began to decline, while in the case of nitrification the greatest accumulation of nitrates took place at the end of the experiment.

There is an inverse relation between ammonia and nitrate in soil. When the nitrifiers become active they convert ammonia to nitrates and as a consequence ammonia almost disappears while the nitrates accumulate. It should be noted that high nitrification followed high ammonification.

Table 11 - Ammonification in non-sterilized, sterilized and re-inoculated sterilized Alberta soils.

(Average p.p.m. ammonia nitrogen on water-free basis)

		Period of incubation.											
		Right after ster.		3 days		6 days		2 weeks		4 weeks		6 weeks	
		Dup.	Ave.	Dup.	Ave.	Dup.	Ave.	Dup.	Ave.	Dup.	Ave.	Dup.	Ave.
Edmonton black park	Non-sterilized	21.3		68.9		68.3		63.1		24.5		11.2	
		21.	21	70.1	70	68.9	69	64.8	64	25.1	25	11.2	11
	Sterilized and re-inoculated			81.2		123.8		158.7		197.4		100.	
				81.2	81	124.4	124	158.7	159	198.6	198	97.	99
	Sterilized	64.		83.5		82.9		84.7		89.9		85.6	
		57.	61	83.5	84	84.1	84	84.7	85	90.5	90	85.6	86
Vegreville black park	Non-sterilized	43.7	44	72.4		8.2		9.3		8.2		3.	
		43.7		72.4	72	8.2	8	9.3	9	8.2	8	3.	3
	Sterilized and re-inoculated			9.9		98.4		122.5		171.7		66.6	
				10.5	10	91.7	95	122.1	122	172.3	172	67.	67
	Sterilized			77.0		73.6		79.4		85.3		84.5	
				76.5	76	75.9	75	79.4	79	85.3	85	83.4	84
Gros Ventre brown prairie	Non-sterilized	51.5	52	17.5		10.5		14.2		12.9		9.	
		51.5		17.5	18	11.1	11	14.2	14	14.0	13	10.	10
	Sterilized and re-inoculated			61.9		81.2		87.0		113.3		124.	
				61.3	62	80.6	81	87.6	87	113.3	113	127.	126
	Sterilized			63.7		61.9		66.6		66.6		58.	
				64.2	64	62.5	62	66.6	67	66.0	66	60.5	60
Fallis gray wooded	Non-sterilized	31.	33	21.1		19.9		12.1	12	12.9		5.	
		34		20.4	21	19.9	20	12.1		23.4	18	5.	5
	Sterilized and re-inoculated			20.4		26.3		42.		51.4		56.6	
				20.4	20	25.7	26		42	52.6	52	55.4	56
	Sterilized	39		26.9		25.7		29.2		31.5		33.	
		39	39	26.9	27	25.7	26	30.4	30	31.5	32	34.	34

Table 11 continued.

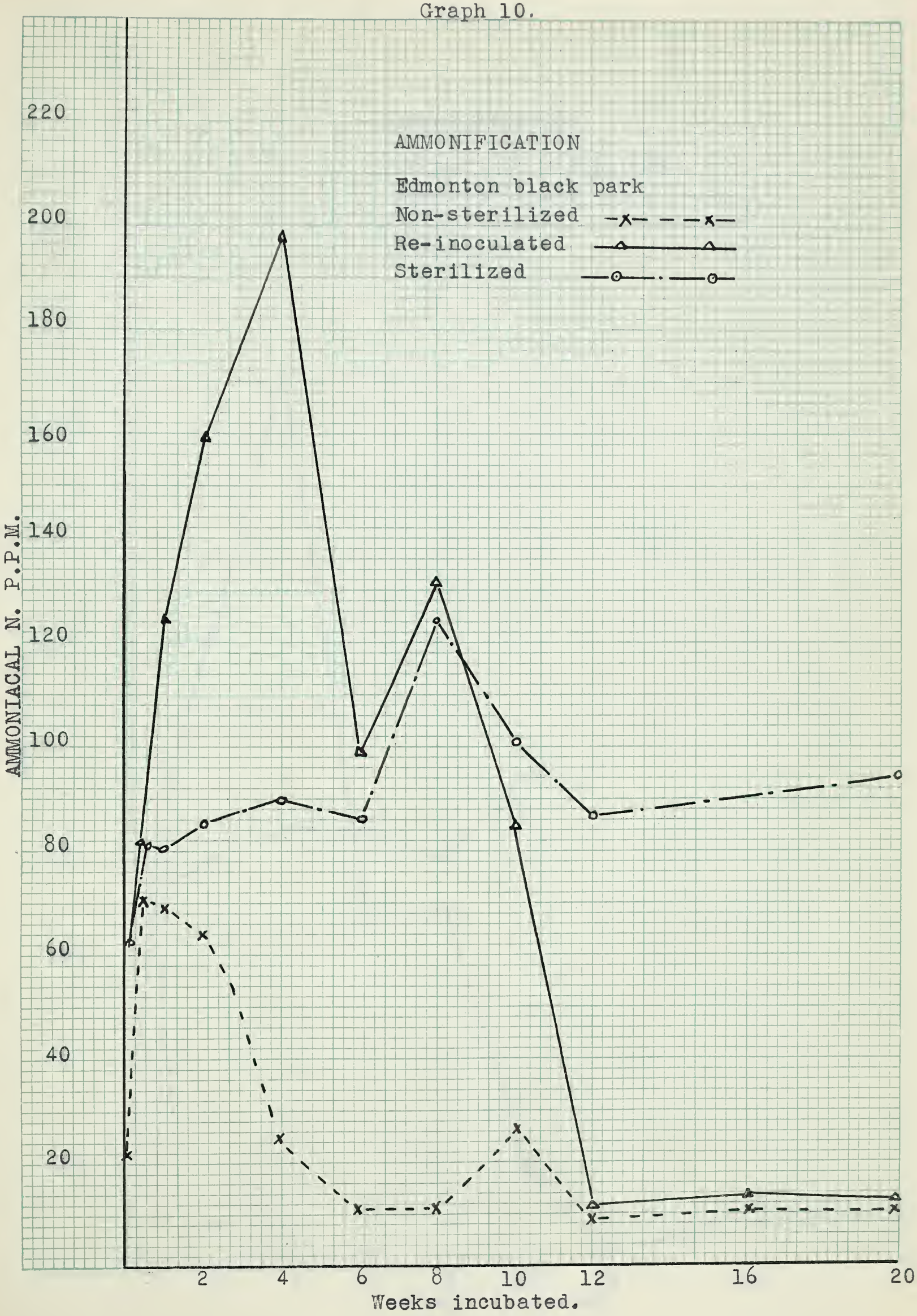
		8 weeks Dup.*	8 weeks Ave.	10 weeks Dup.	10 weeks Ave.	12 weeks Dup.	12 weeks Ave.	16 weeks Dup.	16 weeks Ave.	20 weeks Dup.	20 weeks Ave.	Final Ave.
Edmonton black park	Non-sterilized	11.7		30.2		9.9		9.9		11.7		30
		11.7	12	23.5	27	8.7	9	12.3	11	12.8	12	
	Sterilized and re-inoculated	129.5		79.5		11.0		13.0		11.0		
		134.5	132	90.7	85	11.0	11	15.2	14	14.0	13	92
	Sterilized	130.1		94.0		87.0		----		95.0		
		119.6	125	105.0	100	87.0	87	----		95.4	95	90
Vegreville black park	Non-sterilized	9.3		19.0		3.5		8.2		4.7		
		8.2	9	20.0	20	2.3	3	7.6	8	4.7	5	20
	Sterilized and re-inoculated	69.3		18.0		2.9		11.1		6.4		
		----	69	20.0	19	---	3	10.5	11	9.3	8	50
	Sterilized	79.4		97.4		95.7		----		---		
		77.6	79	90.0	94	95.7	96	----		---		83
Gros Ventre brown prairie	Non-sterilized	14.0		19.0		6.4		12.2		8.2		
		10.0	12	17.0	18	5.3	6	11.1	12	7.6	8	17
	Sterilized and re-inoculated	83.0		22.4		8.2		14.6		11.7		
		84.0	84	22.4	22	7.6	8	11.7	13	11.1	11	61
	Sterilized	117.3		82.0		65.4		66.5		----		
		116.1	117	90.0	86	64.2	65	66.5	67	----		72
Fallis gray wooded	Non-sterilized	15.7		13.4		2.3		7.6		5.9		
		12.3	14	18.0	16	3.0	3	7.6	8	7.6	7	14
	Sterilized and re-inoculated	60.1		61.6		28.6		61.3		9.9		
		57.8	59	64.0	63	28.6	29	60.7	61	10.5	10	42
	Sterilized	33.3		36.0		42.0		30.9		----		
		31.7	33	35.0	36	35.6	39	31.5	31	----		33

*Dup. used to designate the word "duplicates."

Table 21 continued.

| | | |

Graph 10.


AMMONIFICATION
Edmonton black park
Non-sterilized
Re-inoculated
Sterilized
AMMONIACAL N. P.P.M.
220
200
180
160
140
120
100
80
60
40
20
2
4
6
8
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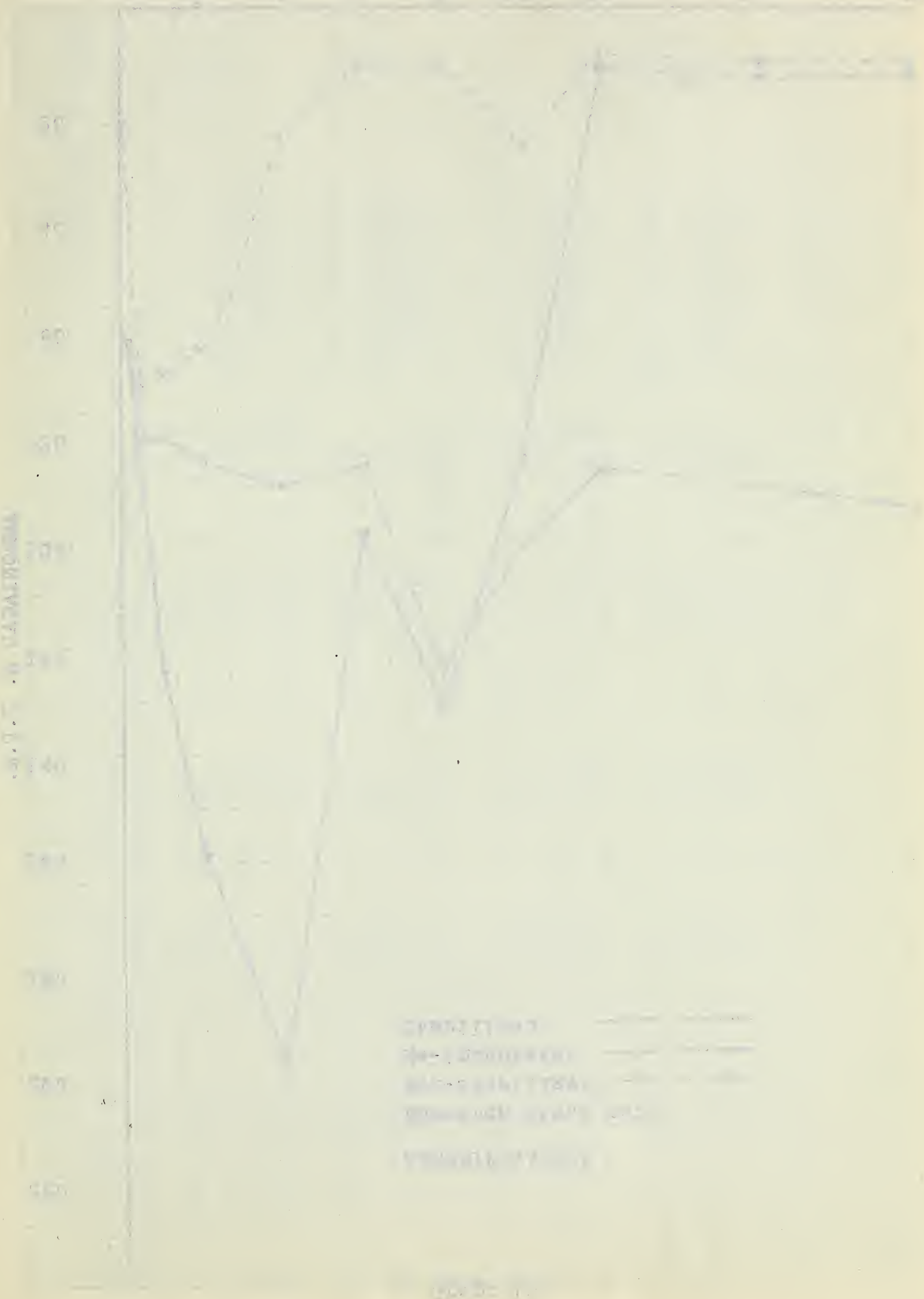
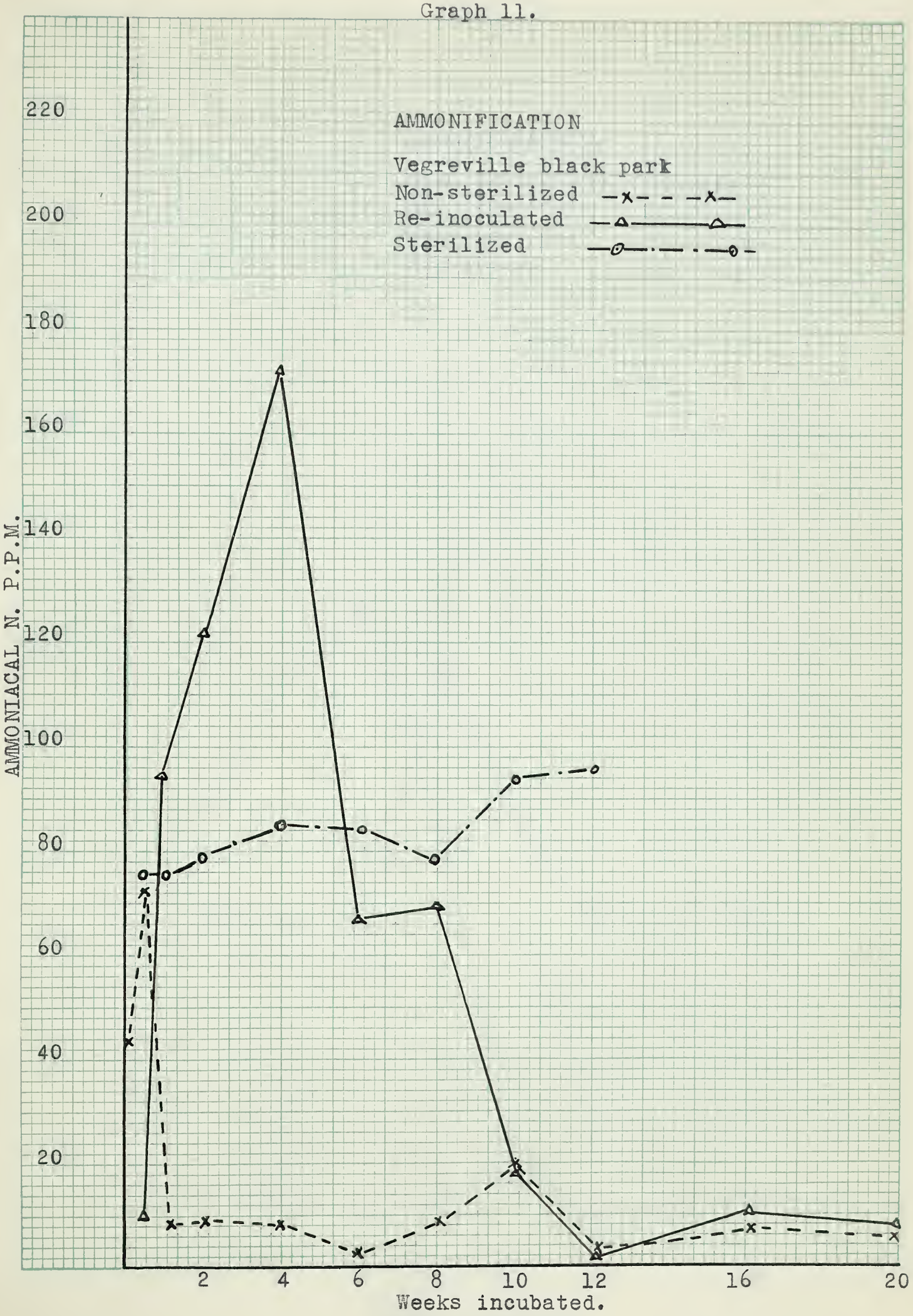
12
16
20
Weeks incubated.



Graph 11.


AMMONIFICATION
Vegreville black park
Non-sterilized
Re-inoculated
Sterilized
AMMONIACAL N. P.P.M.
220
200
180
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20
2
4
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8
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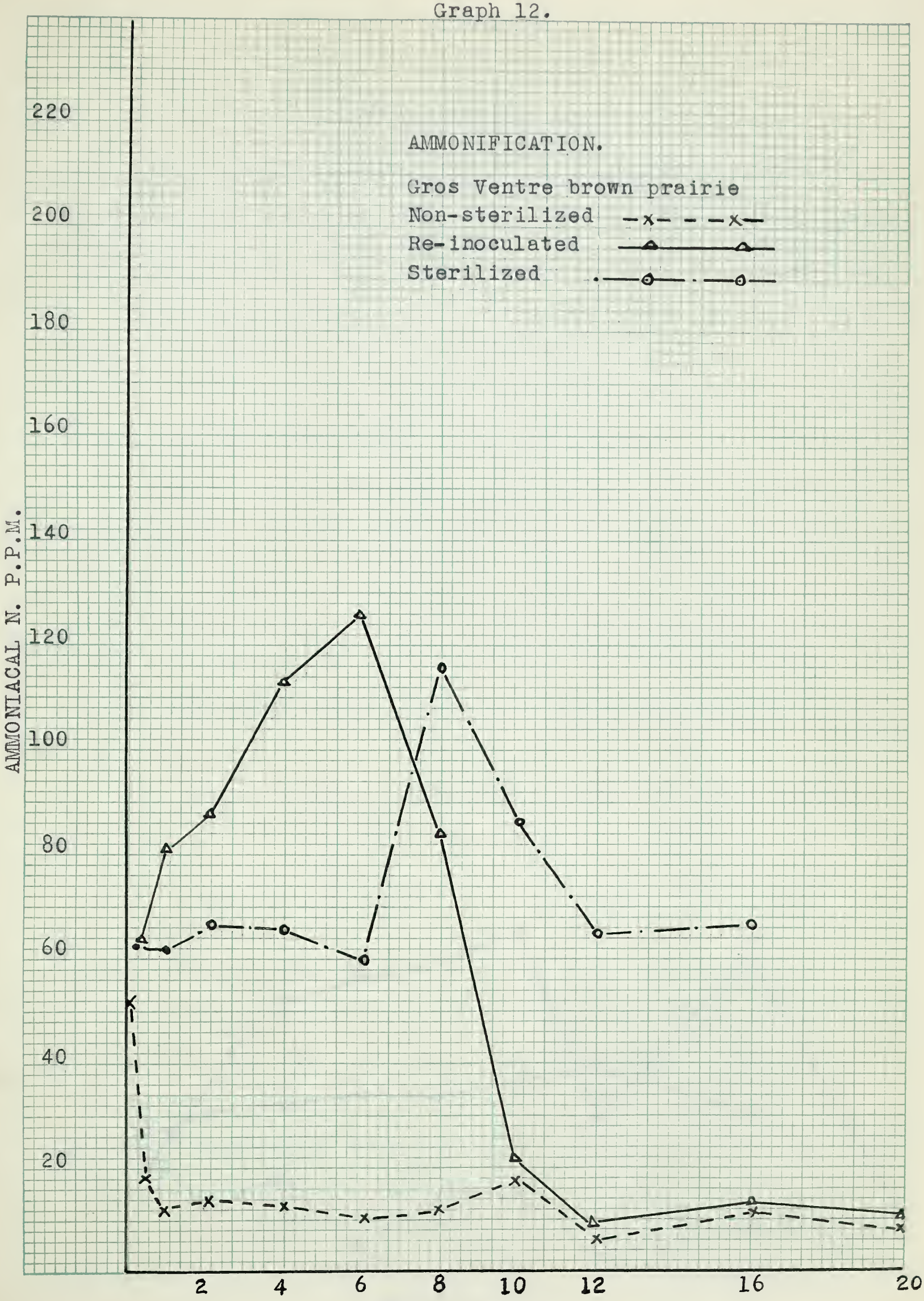
12
16
20
Weeks incubated.

Graph 12.


AMMONIFICATION.
Gros Ventre brown prairie
Non-sterilized
Re-inoculated
Sterilized
AMMONIACAL N. P.P.M.
220
200
180
160
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120
100
80
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2
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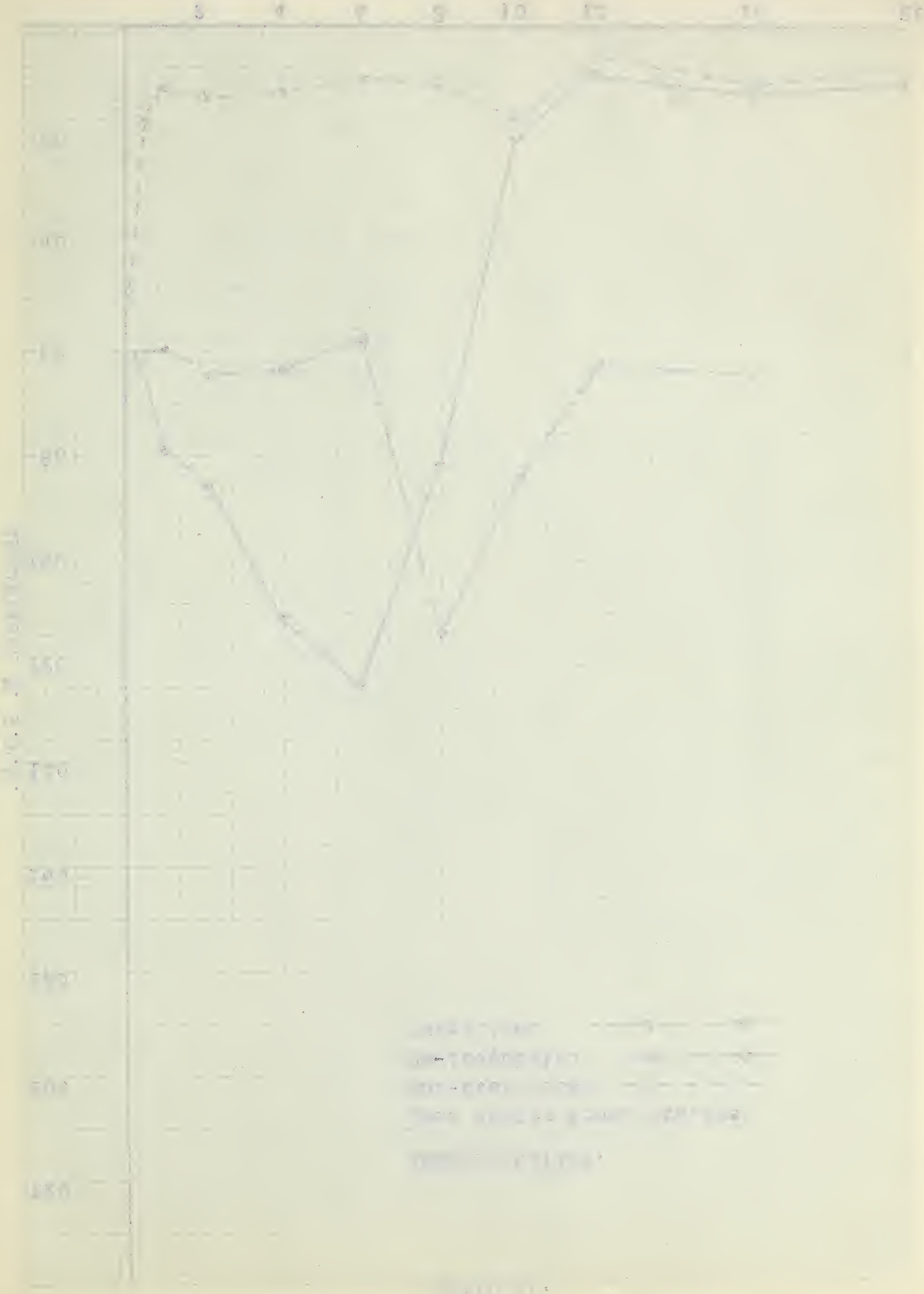
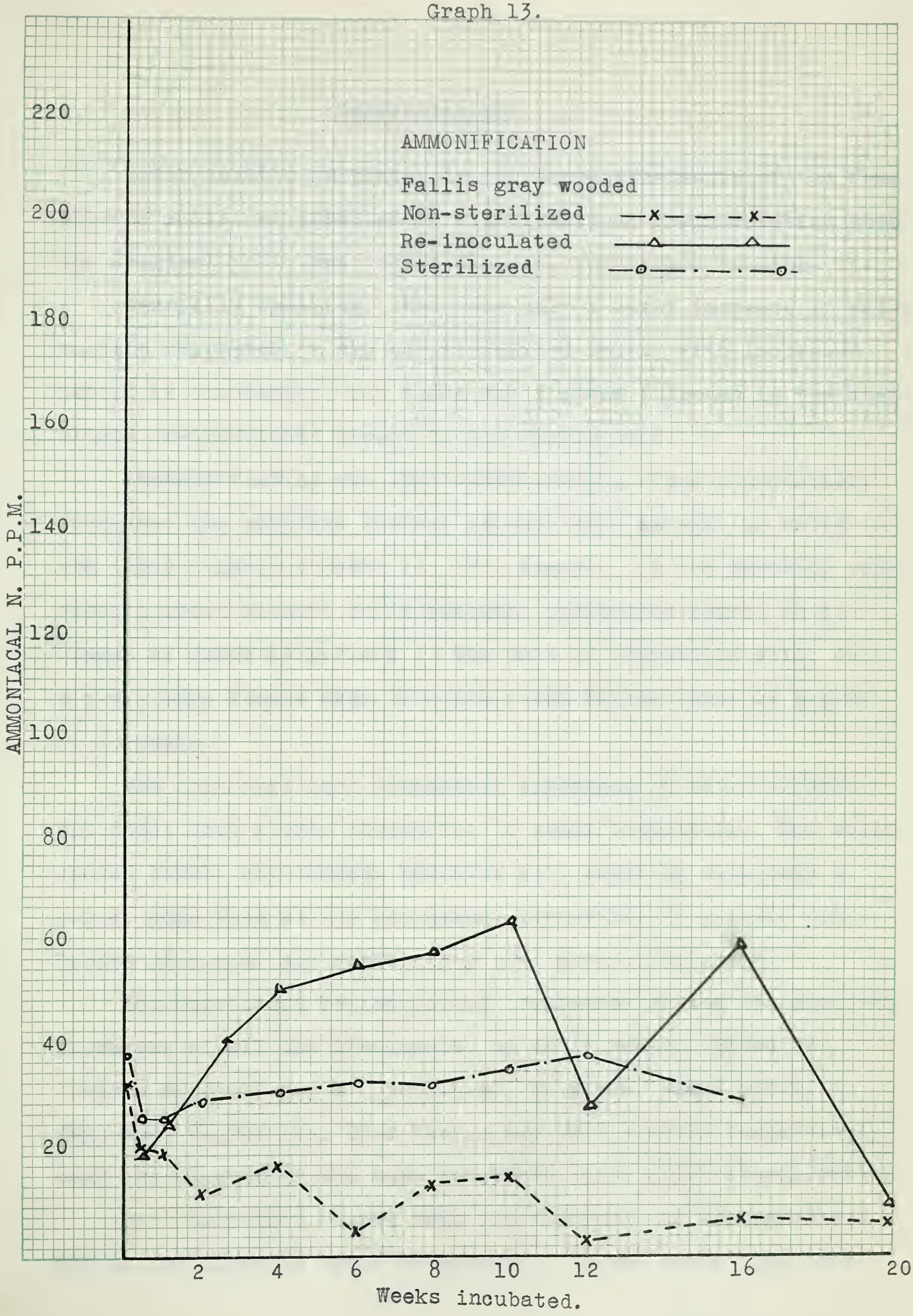
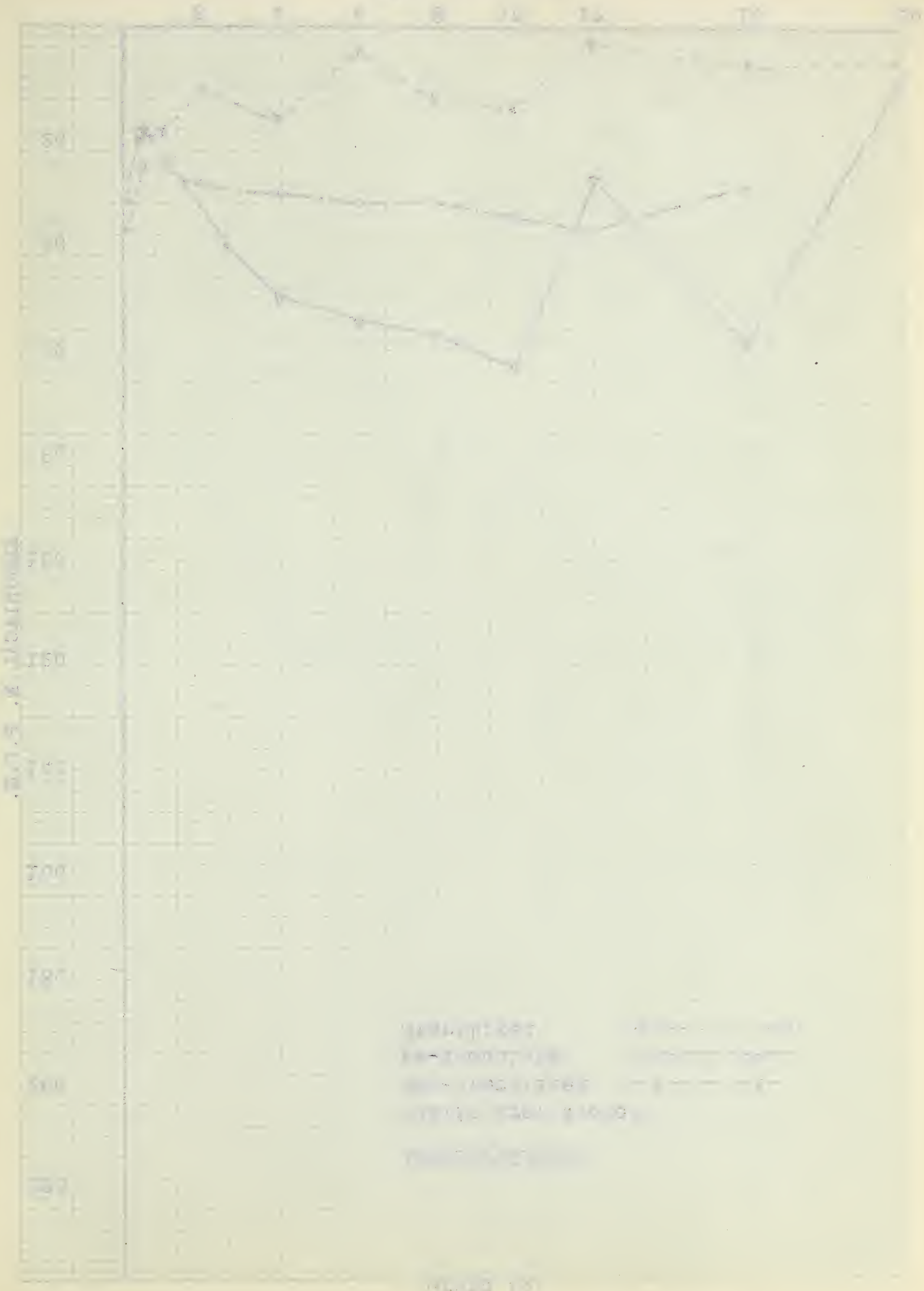
16
20
Weeks incubated.



Graph 13.


AMMONIFICATION
Fallis gray wooded
Non-sterilized
Re-inoculated
Sterilized
AMMONIACAL N. P.P.M.
220
200
180
160
140
120
100
80
60
40
20
2
4
6
8
10
12
16
20
Weeks incubated.



Sulfofication.

Water soluble sulphates in the three treatments of the four Alberta soils, as affected by sterilization, re-inoculation with the original soil, and incubation, are summarized in table 14.

Generally speaking, there was only a small increase in water soluble sulphates in the non-sterilized soils, with increased period of incubation, but there was a great increase in the sterilized re-inoculated except in the Fallis soil.

Another finding was that sterilization alone considerably increased the sulphate content in each soil as will be noted in the first column of table 14. For example, in the Edmonton soil, sterilization without any subsequent incubation caused an increase of about 25 p.p.m.; in the case of Vegreville soil, 46 p.p.m.; Gros Ventre over 30 p.p.m.; and Fallis about 19 p.p.m. of sulphate.

The non-sterilized treatments increased in water soluble sulphates very slowly during the 12 weeks incubation. Two soils showed hardly any change; Edmonton soil ended up with only 8 p.p.m. more than at the beginning, Vegreville 25 p.p.m., Gros Ventre 38 p.p.m. and Fallis 15 p.p.m. more of sulphate.

The sterilized re-inoculated treatments showed the greatest increases of all the treatments, up to 12 weeks. Those increases were 125 p.p.m., 52 p.p.m., 36 p.p.m. and 2 p.p.m. in Edmonton, Vegreville, Gros Ventre and Fallis soils respectively, over the amounts in the same treatment right after sterilization.

The sterilized showed some fluctuations and the agreement was not as close as could be desired. In two soils, the Gros

Ventre and Fallis, sulphates were almost as high as in the sterilized re-inoculated. This was not anticipated and is hard to explain. There is a possibility that some flasks became contaminated; then there is some variation due to sampling, but it would hardly explain the high results obtained.

In Fallis sterilized soil there was almost no increase in sulphates at the end as compared to the beginning of 12 weeks; in Gros Ventre an increase of 16 p.p.m. as judged by the 12 weeks reading but no increase before this; in Edmonton sterilized 45 p.p.m. or about half of that in the sterilized re-inoculated; and in Vegreville about 30 p.p.m. more than at the beginning.

From the results presented in table 14 it appears that water soluble sulphates are increased by sterilization since greater quantities were found in the sterilized and sterilized re-inoculated than in the non-sterilized soils. (There is one exception, that in Fallis soil at 4 weeks incubation, which no doubt was accidental contamination either when evaporating the extract to dryness or in the electric muffle).

Sterilization, therefore, probably causes the breaking down of sulphur compounds in the soil -- chiefly proteins -- making sulphur more easily oxidizable. How it is changed to sulphate form in the sterilized soil where the action of sulphur oxidizing micro-organisms is excluded would have to be explained possibly on some chemical basis such as oxidation. The apparent increase in sulphate in uninoculated sterilized soil may be due merely to an increase in soluble organic matter which on ignition gives higher sulphate results.

In the sterilized re-inoculated soils the sulphur oxidizing bacteria, probably Thiobacillus thiooxidans, Thiobacillus denitrificans, Thiobacillus thioparus, certain ammonifying bacteria (29) and certain sulphur oxidizing fungi would no doubt be responsible for the higher sulphate accumulation.

Either sterilized soil has been made a more favorable medium for these organisms or sterilization has destroyed some antagonistic organisms which if present in the inoculum take a long time to become active enough to bring about an equilibrium such as that which exists in the non-sterilized soil.

Table 13 shows a comparison of the amount of water soluble sulphate found with and without ignition of the evaporated extracts. It shows that without ignition only about one-half as much sulphate was obtained as with the ignition. It suggests that part of the sulphate obtained with ignition comes from the oxidized organic matter and is therefore not precipitated without ignition.

Table 14 - Sulfofication in non-sterilized, sterilized, and reinoculated sterilized Alberta soils.

(Average p.p.m. SO_4 on water-free basis)

		Period of incubation after sterilizing soil and setting up experiment													
		Right after ster.		2 weeks		4 weeks		8 weeks		12 weeks		16 weeks		20 weeks	
		Dup.*	Ave.	Dup.	Ave.	Dup.	Ave.	Dup.	ave.	Dup.	Ave.	Dup.	Ave.	Dup.	Ave.	Final Ave.
Edmonton black park	Non-sterilized	66		59		69		69		82						
		75	71	69	64	62	66	68	69	76	79					
	Sterilized and reinoculated	96		99		103		123		224						
		99	98	106	103	85	94	129	126	221	223					
	Sterilized	97		125		79		130		144						
		95	96	103	114	--	79	118	124	137	141					
Vegreville black park	Non-sterilized	63		65		70		103		103						
		89	76	72	68	--	70	148	126	98	101					
	Sterilized and reinoculated	136		143		100		158		190						
		136	136	103	123	--	100	158	158	185	188					
	Sterilized	123		132		85		151		154						
		120	122	161	147	85	85	96	124	149	152					
Gros Ventre brown prairie	Non-sterilized	40		62				65		72						
		43	42	41	52			114	89	87	80					
	Sterilized and reinoculated	81		67		96		103		108						
		58	70	68	68	75	86	106	105	103	106					
	Sterilized	104		95		86		109		123						
		116	110	109	102	--	86	106	108	128	126					
Fallis gray wooded	Non-sterilized	24		51		93		35		41						
		24	24	33	42	69	81	41	38	36	39					
	Sterilized and reinoculated	51		41		45		48		46						
		48	50	43	42	62	54	65	57	57	52					
	Sterilized	41		48		36		32		51						
		45	43	68	58	--	36	62	47	46	49					

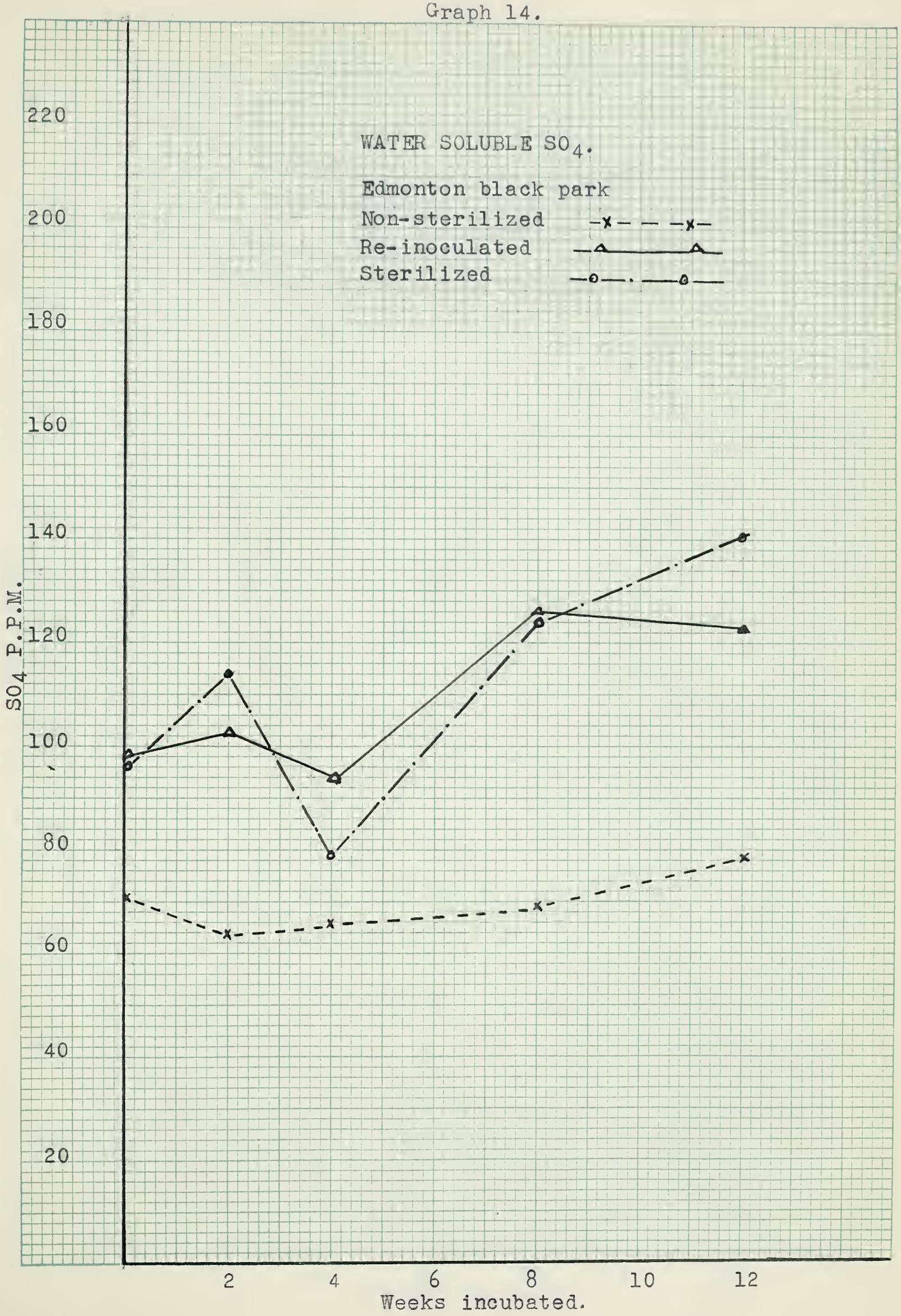
*Dup. used to designate the word "duplicates."

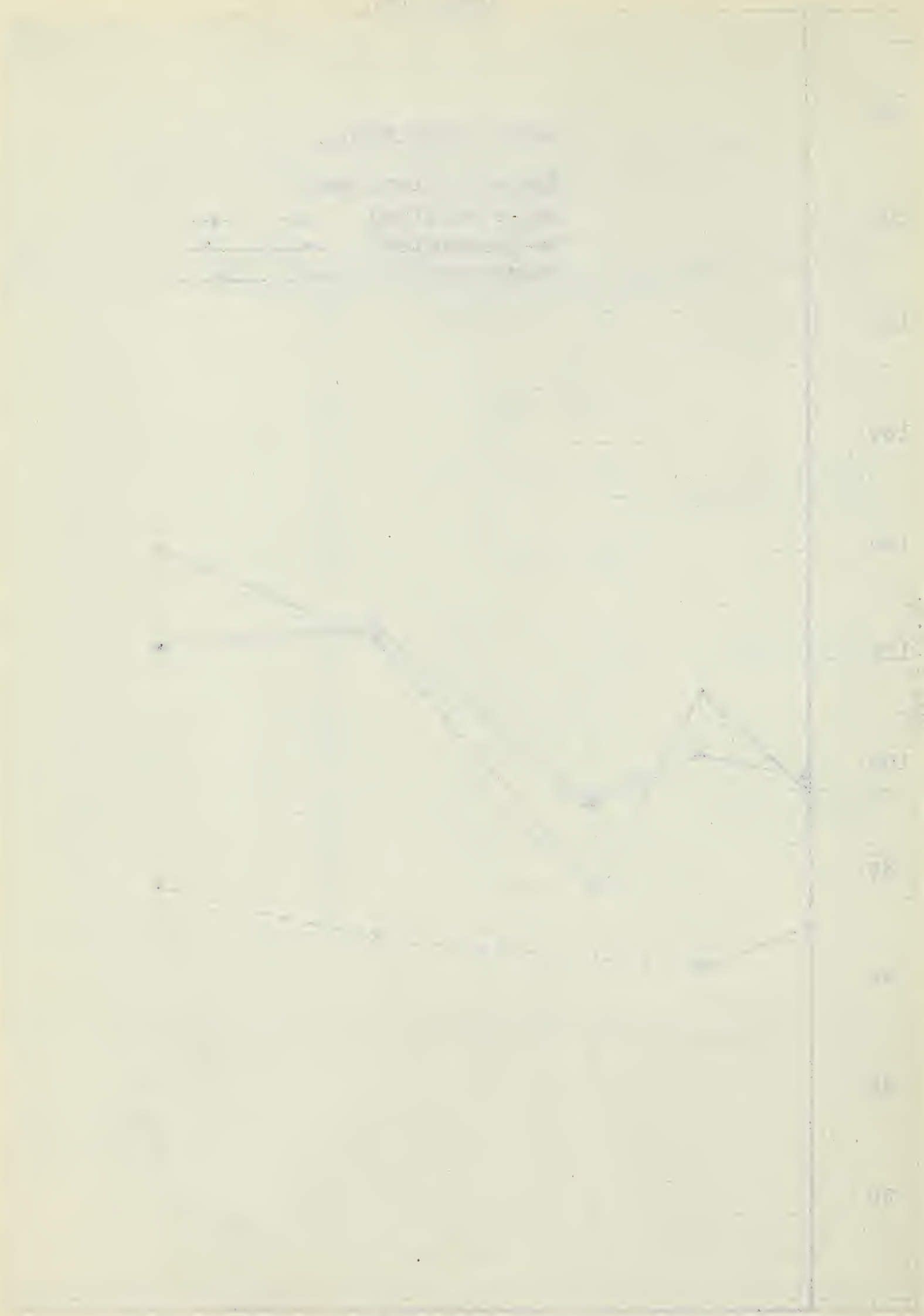
Table 13* - Sulphates with and without ignition.

Soil (incubated 12 weeks)	Treatment	SO_4 p.p.m. Without ignition	SO_4 p.p.m. With ignition.
Edmonton black parkland	Non-sterilized	54	82
		45	76
	Re-inoculated		224
		185	221
	Sterilized	85	144
		78	137
Vegreville black parkland	Non-sterilized	65	103
		63	98
	Re-inoculated	130	190
		125	185
	Sterilized	100	154
		90	149
Gros Ventre brown prairie	Non-sterilized	40	72
		50	87
	Re-inoculated	85	108
		81	103
	Sterilized	70	123
		71	128
Fallis gray wooded	Non-sterilized	30	41
		6	36
	Re-inoculated	20	46
		27	57
	Sterilized	15	51
		15	46

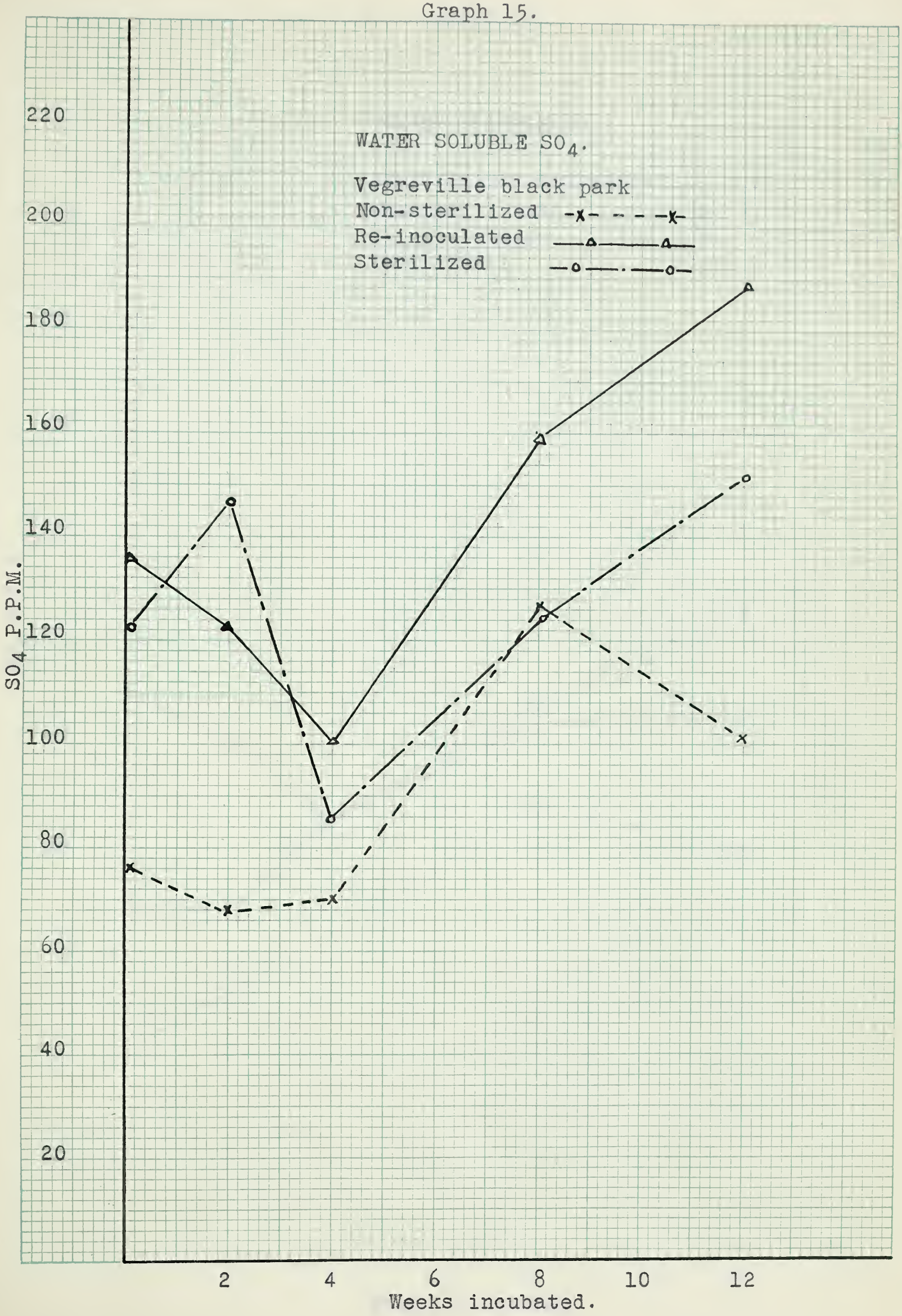
*Table 12 has been omitted as this information has been covered by the first paragraph on page 68.

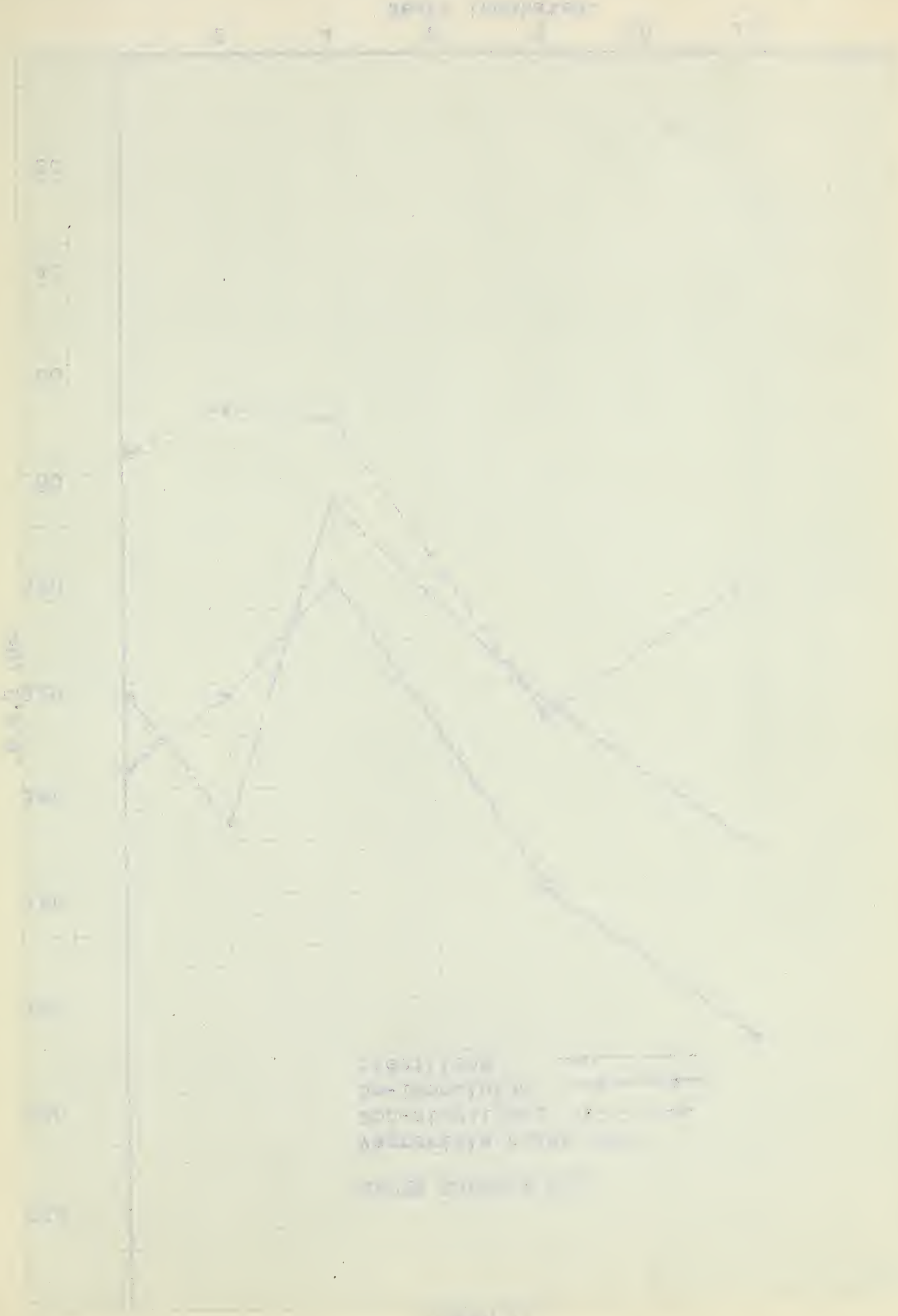
Graph 14.


WATER SOLUBLE SO_4.
Edmonton black park
Non-sterilized
Re-inoculated
Sterilized
220
200
180
160
140
120
100
80
60
40
20
SO4 P.P.M.
2
4
6
8
10
12
Weeks incubated.

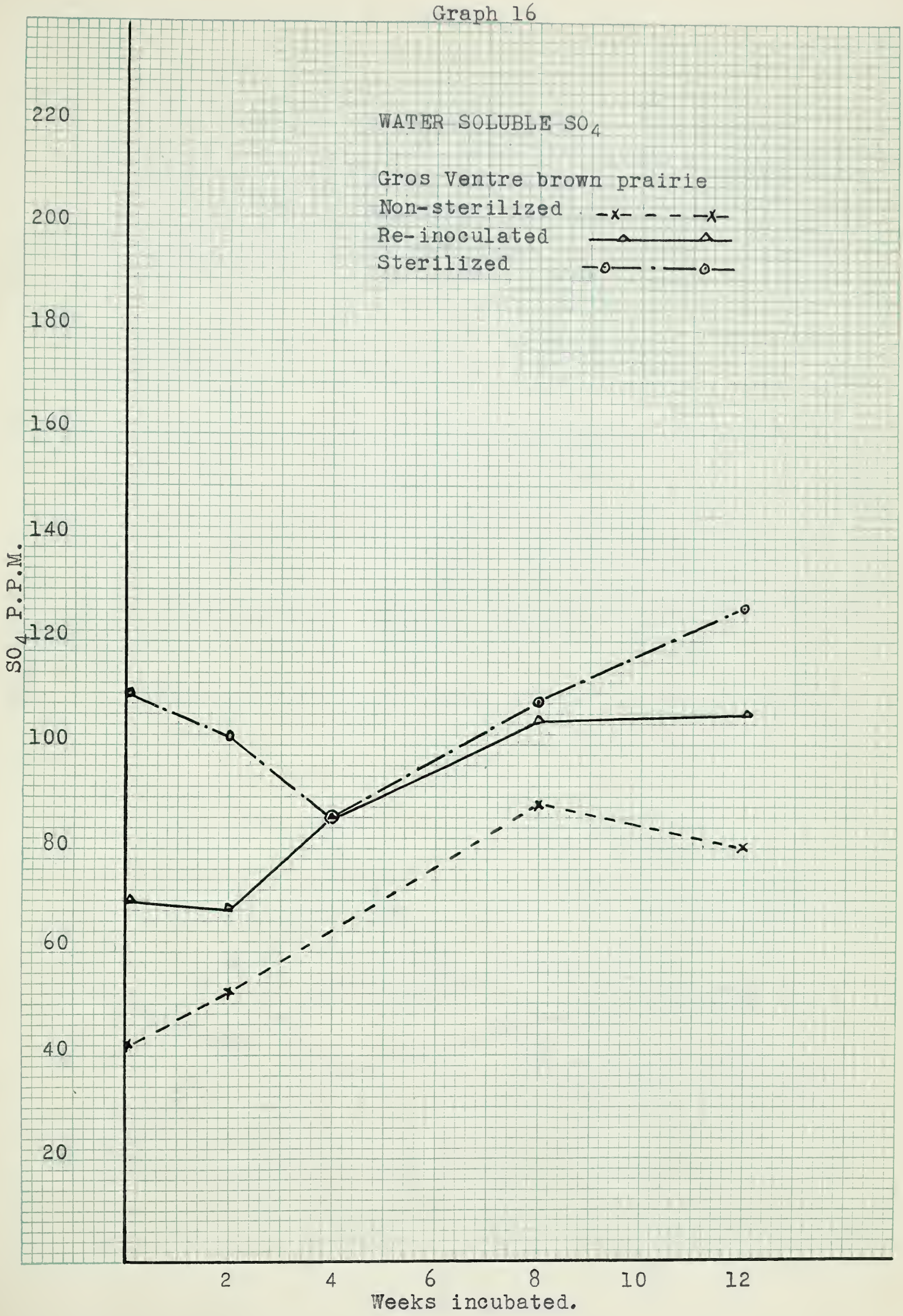


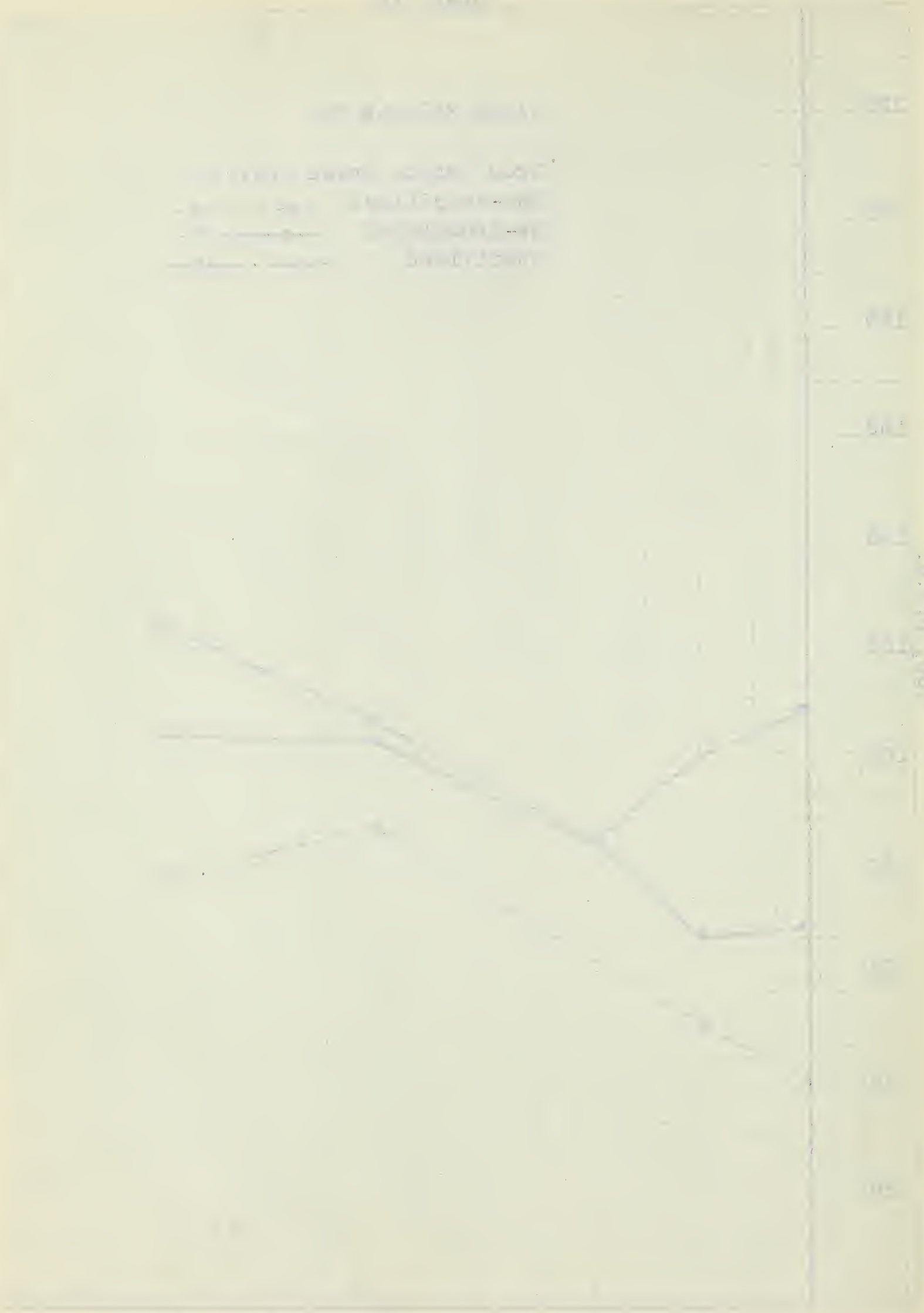
Graph 15.


WATER SOLUBLE SO_4.
Vegreville black park
Non-sterilized
Re-inoculated
Sterilized
220
200
180
160
140
120
100
80
60
40
20
SO4 P.P.M.
2
4
6
8
10
12
Weeks incubated.

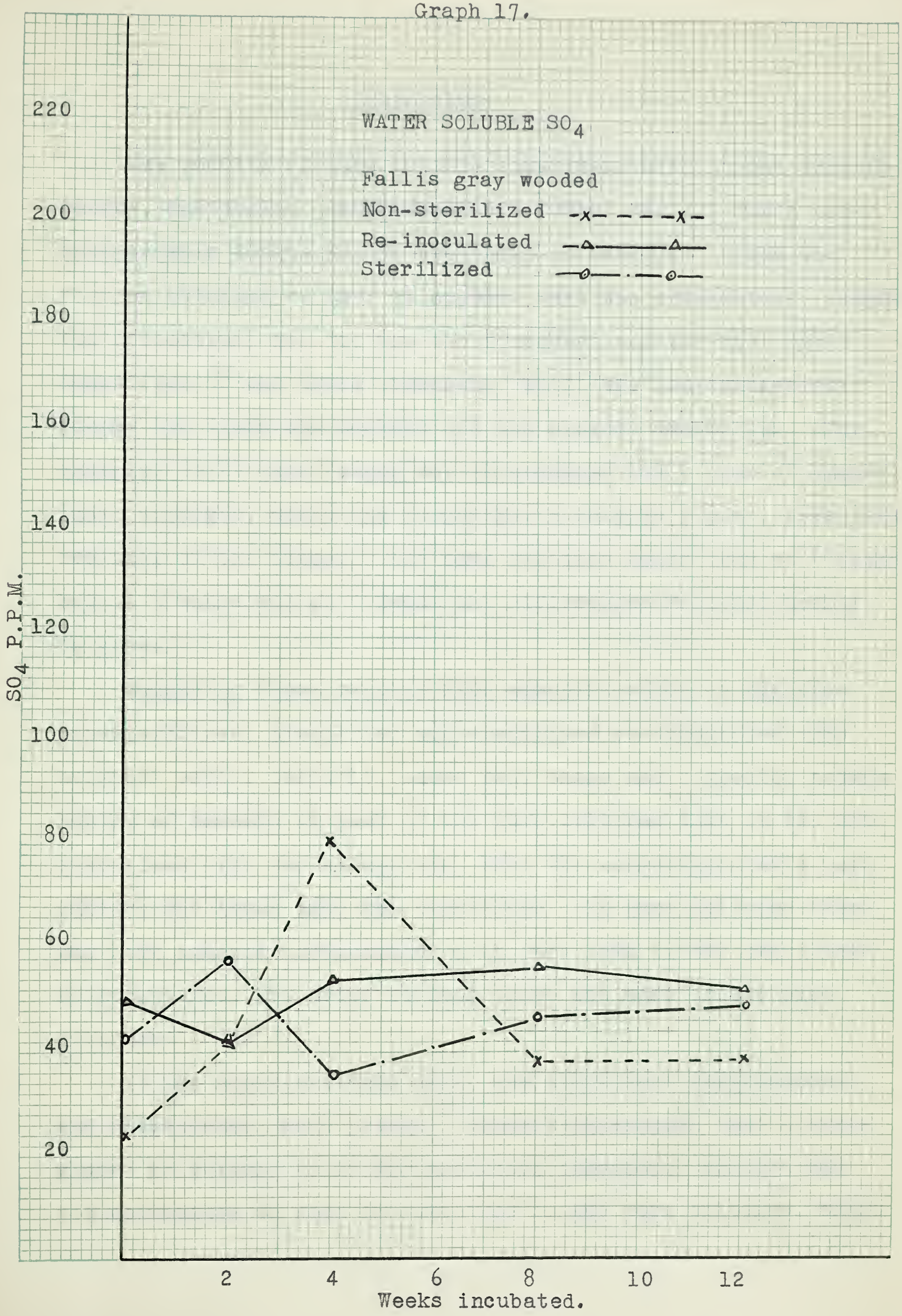


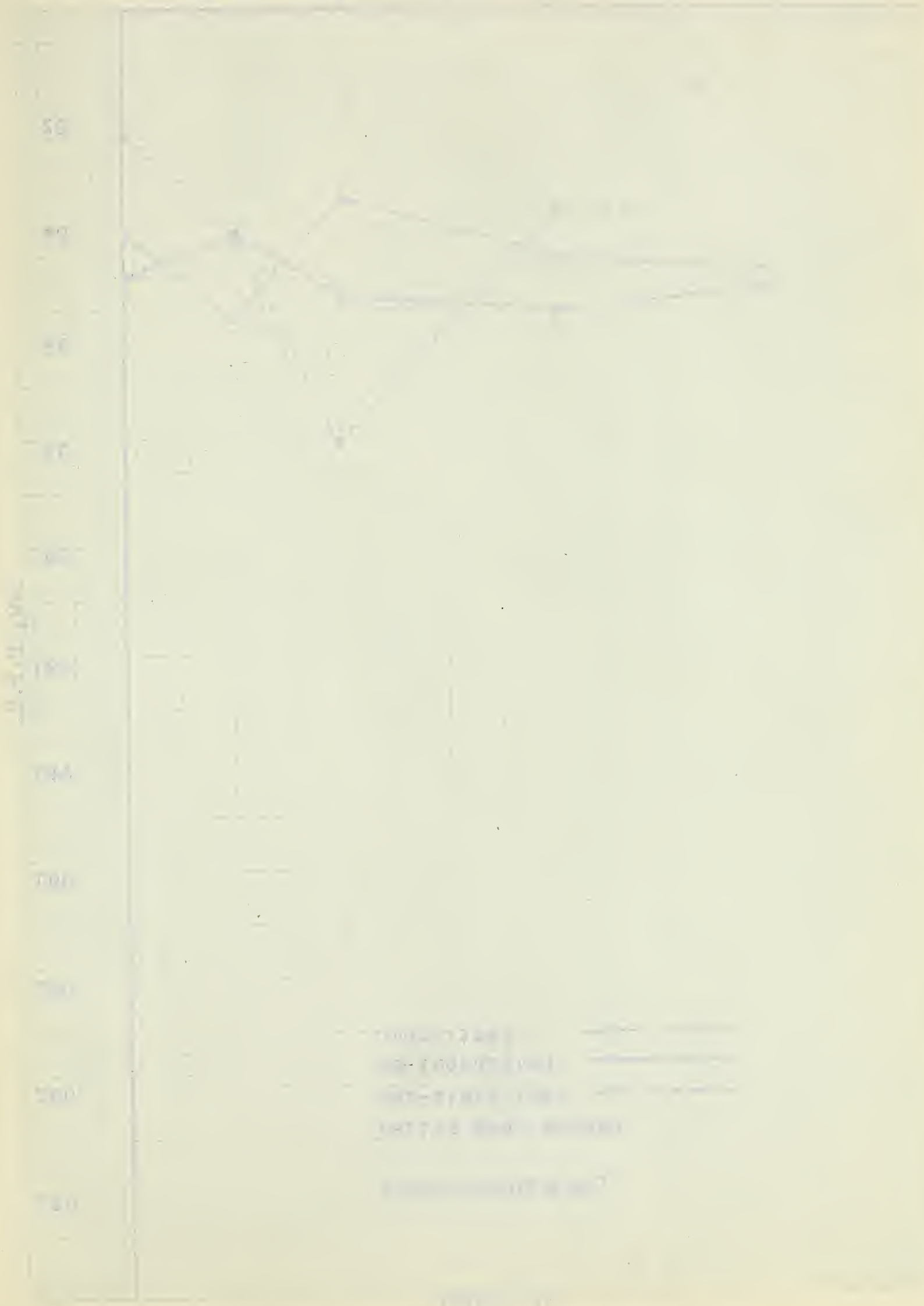
Graph 16


WATER SOLUBLE SO_4
Gros Ventre brown prairie
Non-sterilized
Re-inoculated
Sterilized
SO_4 P.P.M.
220
200
180
160
140
120
100
80
60
40
20
2
4
6
8
10
12
Weeks incubated.



Graph 17.


WATER SOLUBLE SO_4
Fallis gray wooded
Non-sterilized
Re-inoculated
Sterilized
SO_4 P.P.M.
220
200
180
160
140
120
100
80
60
40
20
2
4
6
8
10
12
Weeks incubated.



Ophiobolus.

The results of pure culture experiment with a root rotting fungus, Ophiobolus, an antagonistic fungus No. 32, and a non-antagonistic fungus No. 3, are shown in table 15. From the figures obtained so far, it appears that the antagonistic fungus (to Ophiobolus) No. 32, was the strongest and the most rapid ammonifier of the three organisms, while the non-antagonistic fungus No. 3, was the weakest and the slowest ammonifier. For example, in 15 days fungus No. 32, produced 169 p.p.m. of ammoniacal nitrogen, while the fungus No. 3, only 76 p.p.m., less than one-half of the former. The same relation holds true at 6 weeks, but at 5 weeks and at 4 weeks the differences were not nearly so great.

Comparing these results with ammonification in Edmonton non-sterilized, sterilized and sterilized re-inoculated with original soils, table 15, shows that there was a greater accumulation of ammonia in each of the pure cultures than in the non-sterilized with two exceptions, when the non-antagonistic pure culture was lower than the sterilized. In pure cultures there was less ammonia accumulated in 15 days than in the sterilized re-inoculated, but after 15 days there was more in the pure cultures.

In the non-sterilized soil, the nitrifiers (Nitrosomonas and Nitrobacter) were present, converting ammonia into nitrate almost as quickly as it was produced; therefore, ammonia did not accumulate to such an extent as in the pure cultures, where

the nitrifying organisms were destroyed. In the sterilized soil re-inoculated with original soil, the nitrifiers, killed by sterilization, when introduced again by the inoculum, took some time to become active. Ammonia accumulated at first, but after a few weeks it began to decline; evidently more and more of it was being converted to nitrates. In pure cultures of the three organisms, the nitrifiers were destroyed so that the ammonia was accumulating and not being converted to nitrate.

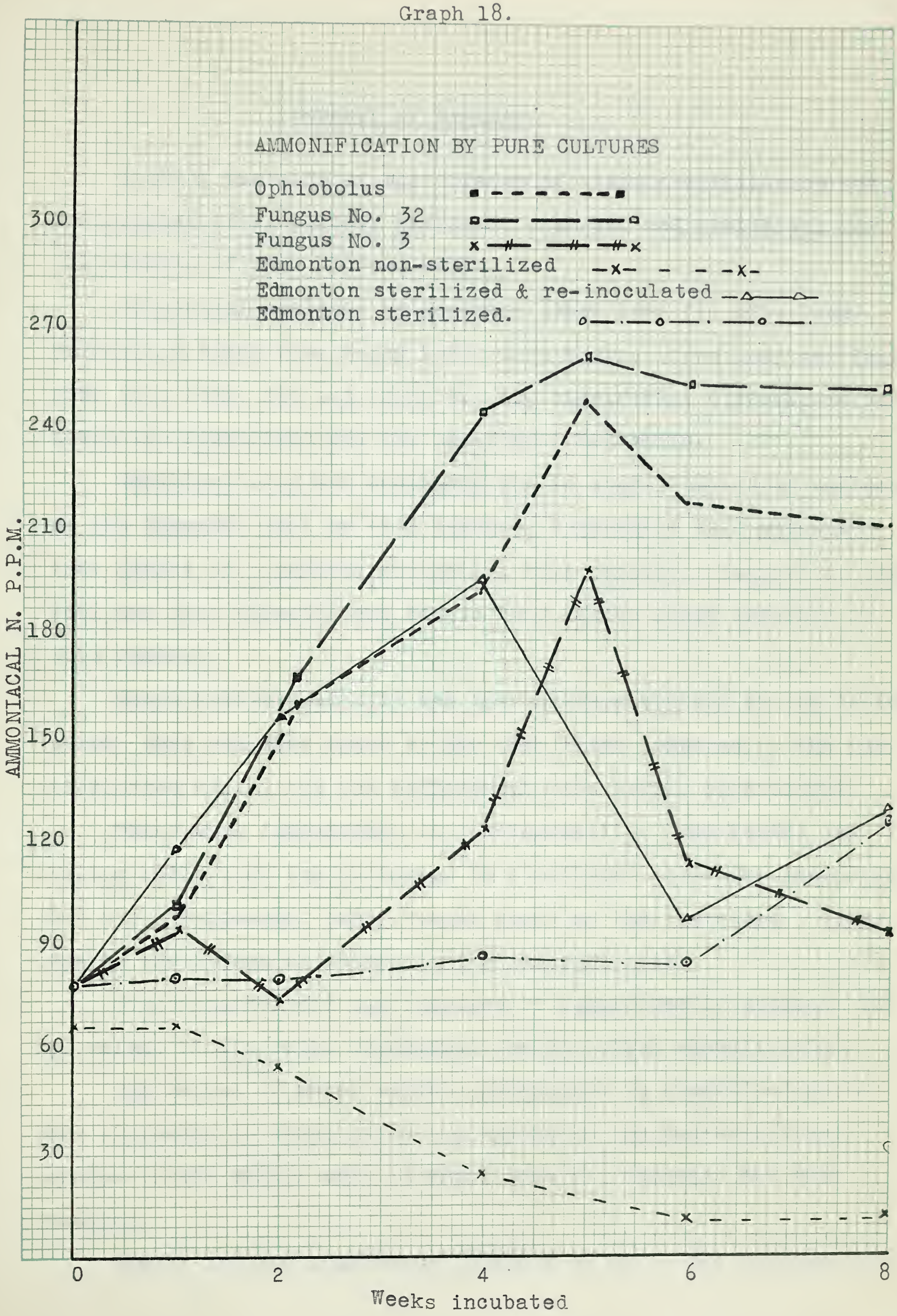
Possible reasons why fungus No. 32, produced more ammonia than the other two organisms are: Firstly, it may naturally be a stronger ammonifier. Secondly, sterilization may have altered the nature of the soil physically and chemically making it a more favorable medium for this particular fungus.

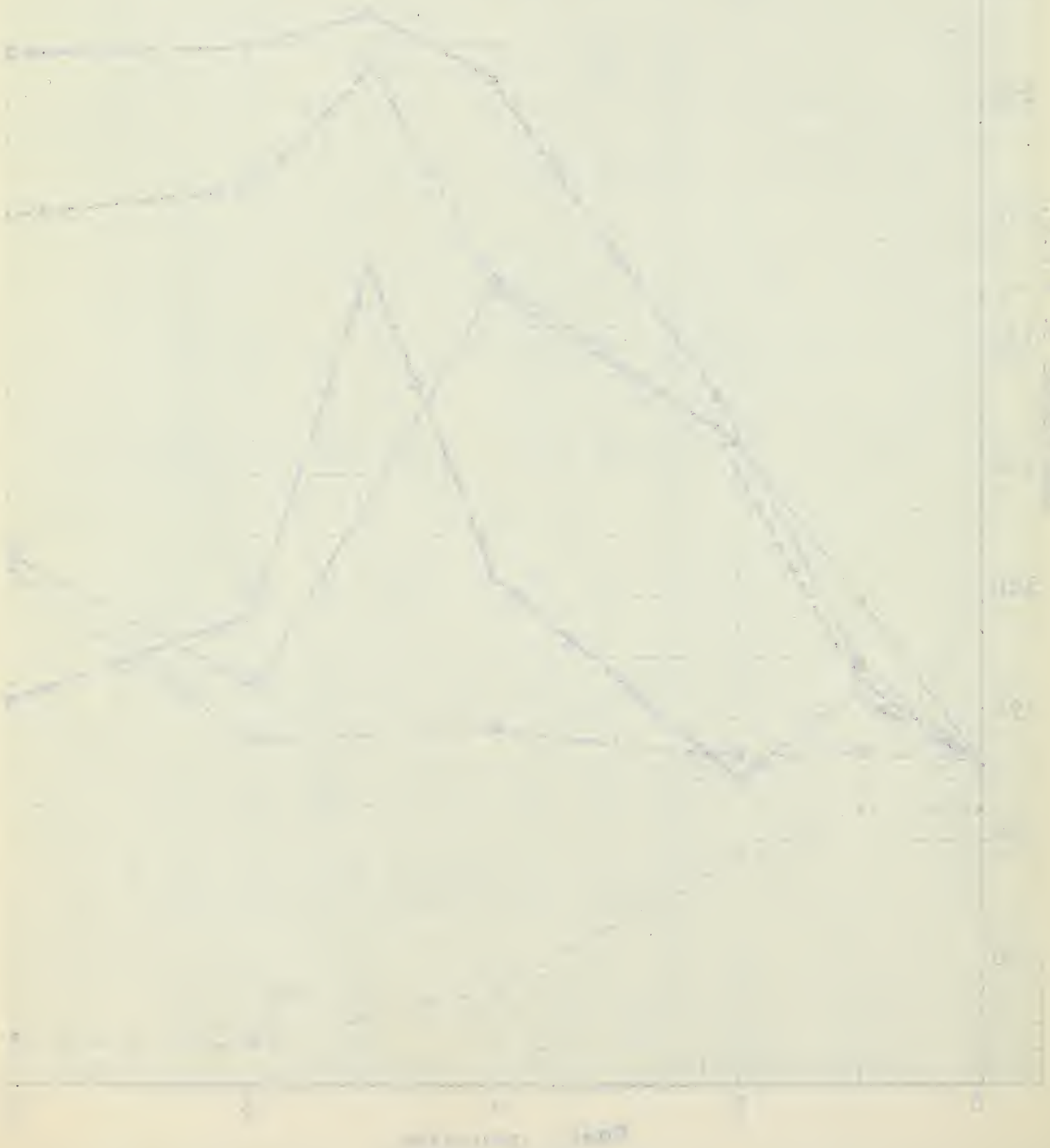
Table 15 - Ammonification by pure cultures of fungi when inoculated on sterilized soil as compared with ammonification in non-sterilized, sterilized, re-inoculated sterilized Edmonton sod.
(Average p.p.m. ammonia nitrogen on water free basis)

Edmonton black park ster. soil re-inoculated with							From ammonification experiment					
Days incubated	Ophiobolus		Antagonistic Fungus No.32		Non-antagonistic Act. No. 3		Non-ster.		Re-inoculated with original soil		Sterilized	
	Dup.*	Ave.	Dup.	Ave.	Dup.	Ave.	Dup.	Ave.	Dup.	Ave.	Dup.	Ave.
7 days	100		93		96		68		124		83	
	100	100	116	104	97	97	69	69	124	124	84	84
15 days	161		167		76		63		159		85	
	162	162	170	169	75	76	65	64	159	159	85	85
4 weeks	189		243									
	203		248									
	190				125		25		197		90	
	204	196	248	246	125	125	25	25	199	198	91	90
5 weeks	253		263		195							
	247	250	262	263	205	200						
6 weeks	220		255		114		11		100		86	
	222	221	258	257	118	116	11	11	97	99	86	86
8 weeks	216		253		94		12		130		130	
	215	216	256	255	98	96	12	12	135	132	120	125

*Dup. used to designate the word "duplicates."

Graph 18.


AMMONIFICATION BY PURE CULTURES
Ophiobolus
Fungus No. 32
Fungus No. 3
Edmonton non-sterilized
Edmonton sterilized & re-inoculated
Edmonton sterilized.
AMMONIACAL N. P.P.M.
300
270
240
210
180
150
120
90
60
30
0
2
4
6
8
Weeks incubated



Summary of Results.

A study was made of the effects of steam sterilization and re-inoculation on certain physical, chemical and biological relationships of four Alberta soils.

Mechanical analysis of Edmonton black loam (to silt loam), Vegreville black fine sandy loam, Gros Ventre brown loam and Fallis gray silt loam, did not bring out any significant differences between the sterilized and the non-sterilized soils.

Capillary rise was decreased due to sterilization in the case of the Edmonton soil by 49.3 percent, Vegreville 62.0 percent, Gros Ventre 53.5 percent and Fallis 37.1 percent. Vegreville black soil was most affected and Fallis gray wooded least affected in this respect.

Water holding capacity and the moisture content at the "sticky point" were somewhat lower in the sterilized compared to the non-sterilized soils, but the differences were hardly significant.

There were indications in the Vegreville, Gros Ventre and Fallis soils that the shrinkage in sterilized soils was increased, but the experimental error was as great as the increases. There was no indication of change in the Edmonton soil.

Sterilization did not increase or significantly decrease the pH values of the soils determined one day after sterilization.

The amount of water soluble phosphorus in sterilized soils was increased to about twice the amount in the non-sterilized except in the Fallis soil in which case the increase was much smaller.

Easily soluble phosphorus (soluble at pH 3) was increased by

about one-third on the average in the Edmonton soil and by one-seventh in the Vegreville. However, in the cases of the Fallis gray wooded soil and the Gros Ventre brown prairie soil, the differences were insignificant. Apparently the steam sterilization affects the solubility of phosphorus in soils rich in organic matter to a greater extent than in other soils.

Nitrification was depressed for about 8 to 10 weeks in the sterilized re-inoculated soils but became much more active from 12 to 39 weeks as compared to non-sterilized, especially in soils high in organic matter. The uninoculated sterilized soils maintained a fairly constant level of nitrates throughout the period.

Ammonification in the sterilized re-inoculated soils increased rapidly for 4 weeks in the black Edmonton and Vegreville soils, and for 6 weeks in the brown Gros Ventre soil, then returned to normal in 8 to 12 weeks and remained at about the same level as the non-sterilized until the end of the experiment. Fallis gray wooded soil (low in organic matter) took a long time to reach its maximum and return to normal, although its peak was the lowest of all the soils.

The water soluble sulphate content of the soils was increased immediately following sterilization. There was also some increase in the non-sterilized, sterilized and sterilized re-inoculated soils upon incubation. In the black Edmonton and Vegreville soils the sterilized re-inoculated increased more than the non-sterilized.

Ammonification tests of pure cultures of the root rotting fungus Ophiobolus, an antagonistic to Ophiobolus fungus No. 32, and a non-antagonistic fungus No. 3, when inoculated into sterilized Edmonton black park soil, showed that the antagonistic fungus No.

32, was the most rapid and the strongest ammonifier, and that the non-antagonistic fungus No. 3, was the slowest and the weakest ammonifier.

Steam sterilization of soils has brought about changes in certain physical, chemical and biological relationships of the four Alberta soils used in this investigation.

Acknowledgement.

The writer wishes to express his appreciation to Dr. J. D. Newton, under whose supervision this investigation was carried out, and to Dr. F. A. Wyatt, Head of the Department of Soils, for their kindness and willing assistance.

Acknowledgement is also due to Dr. Ignatieff and members of the Department of Soils for their helpful suggestions, and to the National Research Council for the financial assistance which made this investigation possible.

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